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Characterization of the common eland (*Taurotragus oryx*) estrous cycle

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**CHARACTERIZATION OF THE COMMON ELAND (*TAUROTRAGUS ORYX*)
ESTROUS CYCLE**

A Thesis
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in
The Interdepartmental Program in
the School of Animal Sciences

by
Parker Pennington
B.S., Louisiana State University, 2007
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ABSTRACT

Three oryx species and nine of eighteen antelope species across three tribes are considered endangered by the IUCN. Though the common eland (*Taurotragus oryx*) is not endangered, it lends itself well to the adaption assisted reproductive techniques due to its large size and calm temperament (Hansen et al., 1985). For these techniques to be used efficiently the details of the estrus need to be defined. Previously, Nowak (1999) proposed the eland estrous cycle to be ~21 days. Specifics of the estrous cycle have yet to be defined and are the objectives of this project. The overall objective is the characterization of the common eland estrous cycle. Specific objectives were to (1) observe behavior peri-estrus (2) determine ovulatory follicle size and (3) produce hormone profiles via RIA. The study animals consisted of two groups of four eland females (n=8) housed as a single bachelorette herd at ACRES. Each group was administered both of two commercial estrus synchronization regimens: Lutalyse[®] protocol (Regimen 1) and altrenogest protocol (Regimen 2). Regimen 1 received PGF_{2α} on day 0 and day 11. Regimen 2 received altrenogest for 7 days and given PGF_{2α} on day 7. Intensive sessions were performed around expected estrus and then repeated at subsequent expected natural estrus: blood samples were taken every 12 hours and ultrasonography was performed every 24 hours until disappearance of a large follicle. HeatWatch[®] patches were applied to detect mounting behavior. Mounts were recorded least often during late morning (0600 to 1200). Homosexual mounting behavior was recorded; all mounts were 2 seconds or less. Ovulatory follicle size was determined to be 7 to 10 mm in diameter and estrous cycle duration was 21 ± 1.6 (±SE) days. A total of 7 of 30 possible ovulations (23.3%) were detected across both regimens and 5 of 15 ovulations following estrus induction (33.3%). Suspected ovulations occurred as frequently on either ovary. Average interval from final PGF_{2α} to ovulation was 69 hours. Progesterone fell in 11 of 15 (73%) estrus inductions. Although further work needs to be conducted to confirm, the parameters defined here should help in the application of reproductive techniques to nondomestic ungulates.

CHAPTER I

INTRODUCTION

The common eland (*Taurotragus oryx*) is an antelope species native to southern and eastern Africa and belongs to the bovidae family and bovine subfamily. They are considered 'conservation dependent' by the IUCN (2002) and can be found in herds of 25 to 70 animals and up to 100 individuals at times. They exhibit crepuscular behavior, grazing and foraging at dawn and dusk. The eland is considered a browser, preferring leaves and shrubs, since grass is not the major component of the diet (Abdullahi, 1981; Codron et al., 2007). They are found mainly in lightly forested areas as well as grass lands of southern Africa. The common eland can be identified by its tan colored coat with spiral horns that are characteristic of its tribe, Tragelaphini. Both sexes display horns and this coat pattern, but females tend to be lighter colored with wider set, thinner horns than the males. Black striping can also be seen around the back of the forelegs and white stripes across the withers and back. Females can stand up to 1.2 meters tall (120 inches) and weigh up to 450 kg; males can weigh up to 2,000 pounds (900 kg) making these, along with the giant eland (*Taurotragus derbainus*), the largest of the antelope species. Indigenous predators of the eland include African lion (*Panthera leo*), spotted hyena (*Crocuta crocuta*) and/or African wild dogs (*Lycaon pictus*). Also native to eastern Africa and sharing rangeland with the common eland are endangered species such as the eastern bongo (*Tragelaphus eurycerus*), mountain nyala (*Tragelaphus buxtoni*) and the giant eland. It is the hope and goal that this project and others like it can and will be used as models for these endangered species to help prevent further loss of these species.

Some aspects of the estrous cycle are known about the common eland, and other species as well, but specifics remain unknown. Estrous cycle length has been monitored and is understood to be ~21 days in the eland (Nowak, 1999; Posselt, 1963). Also, gestation has been monitored and documented to be ~8 months, 271 ± 2.9 days (Dittrich, 1972; Pappas, 2002;

Skinner and Van Zyl, 1969). These parameters have been defined by behavior; either time between standing to be mounted or from last mounting to parturition for estrous cycle length or gestation respectively. Also, they are not considered seasonal breeders, though they exhibit a marked increase in calving during the rainy summer months, January through February, in the wild (Jeffery, 1979; Posselt, 1963). This time period coincides with an increase in available foodstuffs in the southern African grasslands. However, eland do not often exhibit this pattern in captivity and this may be due to the constant availability of foodstuffs within a captive setting. Sexual maturity is reached ~2 years of age for females and 4 years for males, and weaning of calves is ~6 months of age (Hall, 1975; Underwood, 1981). The more specific aspects of the estrous cycle, such as behavior at estrus, size of ovulatory follicle and time to ovulation have yet to be clearly defined.

The common eland lends itself well to the adaption of assisted reproductive techniques such as artificial insemination and embryo transfer due to its body size and relatively calm nature (Hansen et al., 1985). Efforts at domestication of the eland have been implemented (Lightfoot and Posselt, 1977) and the eland has been considered as an alternative meat source (Hall, 1975), though little progress has been made on a large scale. The large size of this species allows for rectal manipulation of the reproductive tract (most other antelope species are much smaller in size) and is immensely helpful for the application of artificial reproductive techniques. Previous efforts have been made at the adaption of domestic cattle techniques within this and other species such as the banteng (*Bos javanicus*; Johnston et al., 2002), guar (*Bos guarus*; Godfrey et al., 1991), blackbuck (*Antilope cervicapra*; Holt et al., 1988), water buffalo (*Bubalus bubalis*; Drost et al., 1983), scimitar-horned oryx (*Oryx dammah*; Pope et al., 1991) and addax (*Addax nasomaculatus*; Densmore et al., 1987). However, even with these advantages, care must be taken when handling nondomestic species as equipment becomes an issue for manual restraint. The use of a hydraulic or drop-floor chute is most often employed when using only manual restraint (Atkinson et al., 1999). Also, the issue of stress is a foremost

concern as the onset of capture myopathy can be and is often fatal to non-domestic species. A single stressful event such as relocating can interrupt physiological processes including ovulation and change of cycle length (Liptrap, 1993; Moberg, 1987). Repeated handling can cause capture myopathy and currently only therapeutic measures can be taken after it has set in, the end result is often renal failure (Spraker, 1993). Keeping all of this in mind, the common eland remains an excellent candidate for the adaption of domestic cattle techniques such as estrus induction and ultrasonography using the proper handling equipment.

It is the overall objective of this project to characterize the common eland estrous cycle for the future use of assisted reproductive techniques both in this species as well as others. The specific aims of the present study are to: (1) determine behavior surrounding estrus, (2) determine ovulatory follicle size and (3) determine the circulating hormone concentration of the common eland estrus after both induced estrus and at natural estrus.

CHAPTER II

LITERATURE REVIEW

The Need for Research of Nondomestic Ungulates

The definition and characterization of nondomestic bovid estrus is needed due to the number of endangered species faced with extinction. Three oryx species were critically endangered this decade. Nine of eighteen antelope species across three tribes are considered endangered by the IUCN. Some species have even been extinct in the wild and have since been reintroduced such as the Arabian oryx (*Leucoryx oryx*). Other species that are endangered include the eastern bongo (*Tragelaphus eurycerus*), mountain nyala (*Tragelaphus angasii*) and western giant eland (*Taurotragus derbianus*). One reason for the current endangered status of nondomestic bovids is unregulated hunting; the Arabian oryx was extinct in the wild due to such practices. Although nearly 600 individuals still persist in captivity problems of breeding management are present. Reintroduction efforts for the Arabian oryx into the wild have begun and a small population now exists in Saudi Arabia (Spalton et al., 1999). Other species considered extinct in the wild are the mohor gazelle and the scimitar-horned oryx (Abaigar et al., 1997; Morrow and Monfort, 1998). Though reintroduction efforts have begun large scale success is limited until the reproductive cycles of such nondomestic species are better understood so breeding efficiency may be improved.

The application of domestic bovine techniques holds the most potential for research and reproductive efforts in nondomestic bovid species. Some techniques, such as somatic cell nuclear transfer and to an extent embryo transfer, are not very efficient. Simpler techniques, such as artificial insemination, hold potential for the assisted propagation of antelope and deer species (Commizzoli et al., 2000). Some advantages are that gametes are easier to collect and transport (sperm) rather than animals (Loskutoff et al., 1995). The inefficiencies experienced by

commercial cattlemen would also be the inefficiencies experienced by those attempting artificial insemination in antelope and deer. Low estrus detection rates are and would be major obstacles for using artificial insemination. While the domestic bovine estrous cycle is understood, that of most nondomestic bovid species is not. Further basic research is needed before any appreciable advancement can be made in the application of assisted reproductive techniques. There is currently a fairly large amount of basic research on estrus duration and basic hormone patterns, some of which will be reviewed in the following section. After a basic understanding of a species' estrous cycle is reached, research to accurately and safely control the estrous cycle is needed to apply techniques commonly used with domestic bovine such as artificial insemination.

Introduction to the Common Eland Antelope (*Taurotragus oryx*)

The common eland antelope is native to southern and eastern Africa. The common eland and the giant eland (*Taurotragus derbianus*) belong to the tribe Tragelaphini, which encompasses the 'spiral horned' antelope. Males can stand up to 163 cm at the shoulder, weighing 500 to 600 kg and females stand up to 142 cm, weighing 340 to 445 kg. Both sexes display a fawn coat color, a pendulous dewlap on the neck and white striping across the withers (Figure 2.1); mature males have coarse dark hair growing between the horns and down the face. Males appear heavily muscled when in good condition. Twisted horns are present on both sexes, females' are often longer and thinner than male's. The terrain utilized is plain and savannah of southern and eastern Africa. They may graze on grasses but the basis of the diet is achieved from browsing brush, shrubs, and low trees (Buys, 1990). They exhibit crepuscular behavior, feeding in the early morning and late evening, when temperatures are cooler (Lewis, 1978). Eland do not maintain territories as other antelope species; however, they do maintain a herd hierarchy (Underwood, 1981; Wirtu et al., 2004). They roam large distances to keep up



Figure 2.1 is a common eland female. Notice twisted horns, fawn coat color and black striping on backs of forelegs. A pendulous dewlap is present in both sexes and females can stand up to 142 cm.

their foraging habits. Eland can be found in herds of 25 to 60, but herds containing up to 100 have been observed.

From observations, females reach sexual maturity around 2.5 years of age while males reach sexual maturity at 4 years of age (Hall, 1975; Hosking and Withers, 1996). The disparity of puberty onset between sexes may be ensuring that calves born in the same year do not breed as it is likely they have the same sire. There seems to be a peak calving season, August to November in southern Africa, but copulation and calving has been reported at all times of the year in captive settings. This time frame of August to November coincides with the wet season of Africa and is the time of greatest food availability (Posselt, 1963). Gestation lasts 271 ± 2.9 days, ~9 months, and eland carry a single calf per pregnancy. This species can live up to 25 years. The observed estrous cycle length, time between copulations, is 21 to 26 days, with estrus lasting ~3 days. Females can exhibit estrus as soon as two weeks after parturition, but rarely conceive during this interval (Posselt, 1963). This may be correlated with the fact that calves are weaned around 6 months of age. In captivity eland reproduce readily, but calf survival to adulthood is unsatisfactory for farming purposes. Due to their size eland could be a useful alternate meat source for South Africa, as well as, alternate dairy source (Lightfoot and Posselt, 1977; Hall, 1975). A concentrated effort at domestication has not been attempted, possibly due to the expense of diet supplementation and low calf survival, but improvements in these areas could be eminent. Advantages to their domestication are low water requirements, large frame, high milk yield and relatively calmer nature than some other antelope species. They have also shown promise when attempts to tame eland have been made. Though it does not appear to be correlated with a herd hierarchy, some eland have shown potential to be conditioned, a possible precursor to domestication (Wirtu et al., 2004). As of 2002 the IUCN classified the common eland as low risk but conservation dependent and there is no immediate threat to the survival of the species, which may be helpful to a domestication effort.

Eland are gender specific in their chromosome number. Males and females contain a diploid number of 31 and 32 chromosomes, respectively. The Bovidae family ranges in diploid chromosome number from 30 to 60 (Gallagher and Womack, 1992). Other species that exhibit this gender specific diploid chromosome number are nyala ($2n = 55$ male/ 56 female), bongo ($2n = 33$ male/ 34 female), bushbuck ($2n = 33$ male/ 34 female) and greater kudu ($2n = 31$ male/ 32 female) (Rubes et al., 2008). In females of these species, one of the X chromosomes is large and acrocentric while the other is late replicating. In males, the Y chromosome is late replicating and translocates to an acrocentric autosome, giving the one less diploid number (Wurster, 1972). Suggestions have been made that this chromosome patterning is an indication of evolution and lineage break in the tregaliphid tribe from the other tribes and genus' of the bovidae family (Rubes et al., 2008; Wallace, 1978). Species within this tribe and exhibiting this chromosome pattern may be acceptable donors and recipients when considering hybrids and interspecies embryo transfer. Previously, an eland x greater kudu hybrid has been produced, though the male offspring is sterile (Jorge et al., 1976). Also, eland have successfully served as surrogate mother to bongo embryos via interspecies transfer (Dresser et al., 1985).

Nondomestic Bovid Estrous Cyclicity

Estrous cycles of several species of nondomestic bovids have been described in length of estrus, as well as, duration of luteal phases. While little information is available on the ovarian activity of several species, most information has been gained from hormonal analysis. Assessment of hormones can come from either of two common methods of sample collection; fecal or urine collection of individual animals, as well as blood sample collection (Ostrowski et al., 2005; Shaw et al., 1994; Metrione et al., 2008; Pickard et al., 2003; Skinner et al., 2001; Thompson et al., 1998). Steroid hormone concentrations can then be determined through either radioimmuno assay (RIA) or enzyme linked assay (EIA). Fecal collection is usually more practical within research facilities and zoos for nondomestic animals due to the reduced stress

levels experienced by the animals (Schwarzenberger et al., 1996). However, with this technique some adjustments must be made for the lag time of gut motility of the animals as well as corresponding fecal samples to correct individuals can be problematic. In addition, most studies to monitor estrous cycle via fecal collection are conducted in captivity. Determining the reproductive status of individuals in the wild by fecal collection has been done, but the inherent difficulties involved have limited its application. The effectiveness of fecal collection of non captive individuals to assess hormonal status has yet to be effectively determined. However, Ostrowski et al. (2005) determined pregnancy in free ranging Arabian oryx throughout 1 year successfully. This begins to demonstrate fecal collection as a reproductive status indicator as well as ovarian activity (Ostrowski et al. 2005). In comparison, blood sample collection requires special facilities such as chutes and experienced personnel to aid in collection. Additional stress is also a consideration for blood collection due to the additional handling.

No matter the method of collection, hormones may be assayed to determine progesterone levels and help understand the whole estrous cycle. Fecal progesterone levels have been used as cyclic activity indicators in a number of species including the scimitar-horned oryx (*Oryx dammah*), Arabian oryx (*Oryx leucoryx*), Jackson's hartebeest (*Alcelaphus buselaphus jacksoni*), wood bison (*Bison bison athabascaae*), mohor gazelle (*Gazella dama mhorri*), and the sable antelope (*Hippotragus niger*) (Ostrowski et al., 2005; Shaw et al., 1994; Metrione et al., 2008; Pickard et al., 2003; Thompson et al., 1998). The springbok (*Antidorcas marsupialis*) and common eland (*Taurotragus oryx*) are species that have been characterized via blood sampling (Skinner et al., 2001; Pennington et al., 2009).

When considering fecal analysis for monitoring ovarian function, such factors as gut motility and lag time for passage of excreta containing steroid metabolites must be accounted for at the end. The appearance of steroid metabolites in the excreta usually takes ~ 12 to 24 hours in ruminates while it only takes ~ 5 hours for them to appear in urine and their presence is

nearly instantaneous in the blood plasma (Schwarzenberger et al. 1996). Due to the entrance of bile at the foregut (ruminants) the steroid metabolites take longer to pass through the gastrointestinal tract than nonruminant species. Other factors to account for are the digestibility of the forages ingested and volume of water ingested, both of which can affect the rate of passage. Radio infusion studies have indicated that bile is the main mode of introduction of steroid metabolites into the digestive tract. They have also indicated that radioactively labeled steroids present in the blood plasma are conjugated and enter the bile and urine rapidly and are then de- conjugated in the intestine. This leaves the excreta with more free metabolites than conjugated. The fecal progesterone is metabolized to a number of series (5- α and 5- β reduced pregnanes). The number of metabolites and type of series varies between species (Schwarzenberger et al., 1996). Due to the lag time between progesterone presence in the blood plasma and its excretion from the body, this method would not be suitable for the stage of the estrous cycle. The exact stage of the estrous cycle could be determined via blood collection and serum or plasma assay. This method has been successful in two species that have been characterized via blood sampling, the springbok and common eland (Skinner et al., 2001; Pennington et al., 2009).

In addition, it has been hypothesized and noted that some nondomestic bovids exhibit a similar estrous cycle to their domestic counterparts (Solti et al., 2000). While total estrous cycle length may vary slightly, the general pattern of falling progesterone prior to estrus, an LH surge, and rising progesterone after estrus has been recorded. To date, estrus has been determined by behavioral observations or copulation if housed with a male or homosexual mounting in some cases confirmed with comparison to progesterone patterns. Many species of nondomestic ungulates have similar estrous cycle lengths to the domestic cow such as the lesser kudu (*Tragelaphus imberis*) (21 to 22 days; Vahala, 1992), common eland (21 days; Posselt, 1963), scimitar- horned oryx (24 to 25 days; Durrant, 1983; Shaw et al., 1994), dik-dik (*Madoqua gunther*) (21 days; Robeck et al., 1997), suni antelope (*Neotragus moschatus*) (21 days;

Loskutoff et al., 1990) and Jackson's hartebeest at 21 to 22 days (Metrione et al., 2008). Little research and information is available on the bongo (*Tragelaphus euryceros*), but estrous cycle length has been documented to be 21 to 23 days (Nowak, 1999) though Mikota et al. (1999) found the length to be 23 to 24 days. Atkinson et al. (1999) also found the common eland estrous cycle to be 23 days. Some dissonance exists between findings, thus further research is clearly required to more precisely define these findings. Seasonality of these species has been a question and topic of debate for some time. A variety of factors contribute to such a mechanism that may be overcome in captivity such as food and water availability, male presence, photoperiod, hemisphere and herd hierarchy (Wirtu et al., 2004; Shaw et al., 1994; Metrione et al., 2008; Pickard et al., 2001; Skinner et al., 1969). Various species have been observed and recorded copulating and calving throughout the year, yet there is a marked increase in calving during the wet season, when food is most available.

Some studies have shown that species such as the springbok, Jackson's hartebeest and lesser kudu (Skinner et al., 2001; Metrione et al., 2008; Vahala, 1992) seem to exhibit a synchronized calving time if not seasonal ovarian cycles. The springbok seems to exhibit an annual anestrous period, though it is classified as an aseasonal breeder. It was determined that cycles ceased in eight ewes from November to April, restarting spontaneously in April. Cycles then ceased again around January for six ewes while the other two did not stop cycling. The six that had ceased restarted again spontaneously again in June. Observations of this species in the wild suggested that the springbok is an aseasonal breeder, but this evidence suggests that from an endocrine view they are seasonal, becoming anestrous 4 to 5 months of the year (Skinner et al., 2001). In this aspect the springbok seems to be similar to the domestic horse or goat. It was evident that some individuals of springbok continued to cycle throughout the year while the majority cease for some time period that is year dependent. It is generally understood that the horse exhibits an annual anestrous period during the 'short days' of the year, the goat is photoperiod dependent as well, though it is considered a 'short day' breeder. The springbok

mechanism has not been conclusively determined but speculated to be ambient temperature (Skinner et al., 2001).

Very few species have been characterized for ovarian functionality and pattern by other means than progesterone analysis. The size of the animal is the main deterring factor that prevents further quantification of the ovaries, such as ultrasonography. One species has successfully been characterized with ultrasound, the wood bison, and the common eland is another that will be discussed in detail in later chapters of this paper. The wood bison is indigenous to Canada and is its largest land mammal (Othen et al., 1999). This larger size contributes to the ability to ultrasound the ovaries. The common eland is the largest of the African ungulates, smaller only to the giant eland. The large frame of this animal also contributes to successful rectal manipulation. Further detail will be given on this topic in later sections.

Artificial Reproductive Techniques in Nondomestic Bovids

One of the major problems associated with breeding exotic antelope in captivity is the prevention of inbreeding depression and maintaining genetic variability. Along with this problem is the difficulty finding genetically appropriate pairs and enabling them to mate. Without the use of assisted reproductive techniques (ART) animals must be shipped to each other, which proposes a significant amount of stress to the animal, elevated cost, as well as, potential spread of disease (Pukazhenthil and Wildt, 2004; Morrow et al., 2009). There are consequences to stress that include the development of capture myopathy and even death in these nondomestic animals. It is for this reason, the reduction of stress and reduction of cost and control of disease that ART would be so useful in the nondomestic field. The ability to ship semen or embryos between institutions would alleviate some of the problems.

Another aspect associated with most antelope species is the variability that exists between species, most of which are indigenous to Africa and surrounding territories. While a similarity in indigenous areas would also seem to mean a similarity in reproductive cycles that is not always the case. As has been discussed, the estrous cycles of most antelope species has been superficially defined (length) but not described in detail. Without the knowledge of impending estrus and duration the efficiency of ART would be severely impaired. Also, one of the primary limiting factors for the use of ART to the domestic industry is the inability to correctly and efficiently detect estrus. Therefore, the estrous cycles of the species intending to benefit from ART must be clearly defined before any success achieved.

Antelope are within the Bovidae family which lends itself to transferring the techniques used in domestics to nondomestic species. However, challenges include the smaller stature of most of the species. This makes such techniques as artificial insemination and embryo transfer that usually require rectal manipulation of the cervix much more difficult. This could however, be overcome by the use of such tools as a speculum like that used in artificial insemination in cervids. Also, the handling systems and general practice used in deer operations would be useful models for antelope practices; deer have many of the same stress problems that antelope do and the specifics of operation, nearly to the commercial level, have been achieved in white tail and red deer. It is also hopeful to aspire to since deer have reached a 50 to 80% success rate with artificial insemination (Morrow and Monfort, 1998). In addition to the smaller stature of most antelope species, each species presents a different reproductive morphology that may impose challenges when performing ART. The scimitar-horned oryx and the addax are the best examples due to the complex, bifurcated nature of the cervix (Morrow and Monfort, 1998).

Institutions that could benefit by the implementation of ART would be zoos, private game owners and research institutions to better utilize the animals they have and to keep their

populations genetically viable without the need to trans-locate animals to obtain suitable pairs. Of course in order for these places to benefit from the use of ART they must participate in the experimentation of such techniques. There has been limited success of artificial insemination and embryo transfer in antelope; however, the repeatability of such success has been low. The low number of animals used in experiments of this nature makes it difficult to consistently conclude findings. Therefore, the joint effort of the institutions mentioned, each housing a low number of animals, is crucial for the success and efficiency of ART to be utilized.

Artificial Insemination

One of the major advantages to artificial insemination (AI) is the near limitless genetic availability from animals both in captivity as well as in the wild (Holt et al., 1996; Wildt et al., 1997). Semen collection and cryopreservation of semen has been routinely performed with consistent success and acceptable semen quality fresh and post thaw with varying freezing protocols (Merilan et al., 1978; Seager et al., 1978; Densmore et al., 1987; Garde et al., 2003). Collection in the field from anesthetized males remains a challenge to keep the sperm viable (Roth et al., 1999), but achievement of satisfactory methods would greatly improve the possibilities that artificial insemination holds. AI also presents a means to spread genetics of fewer males to more females and eliminates the need to remove an individual from the wild population. Electroejaculation has been successfully used in captivity for semen collection (under anesthesia) and can be used for collection in the field (Morrow et al., 2009; Roth et al., 1999).

Crucial to the success of artificial insemination is the detection of estrus (Comizzoli et al., 2000; Morrow et al., 2009). As mentioned, the estrous cycles of some antelope species have been loosely defined (Pukazhenthi and Wildt, 2004). In addition, captive situations do not usually allow for accurate detection of estrus due to a lack of contact with a male. Estrus behavior of females still has yet to be accurately described. The length of the estrous cycle in

several species has been determined including the eland (~21 days), bongo (*Tragelaphus euryceros*) (23 to 24 days), blackbuck (*Antilope cervicapra*) (17 days), addax (*Addax nasomaculatus*) (32 days) and scimitar-horned oryx (24 to 25 days) (Pope et al., 1991). Control of the estrous cycle in several species has been demonstrated including, but not limited to the sable antelope (Thompson and Monfort, 1998), scimitar-horned oryx (Morrow and Monfort, 1998) and common eland (Schwiewe et al., 1990; Nowak, 1999; Pope and Loskutoff, 1999; Pennington et al., 2009). The transfer of protocols from cattle estrous cycle control has had success in the control of nondomestic ungulates, though modifications must be made in the administration of exogenous agents. The use of prostaglandins and altrenogests have been some of the agents used in estrus control with variable results (Pukanzhenti and Wildt, 2004; Comizzoli et al., 2000).

Artificial insemination has been successful in several species although efficiency remains low. Though the number of live births as a result of AI remains low (Morrow et al., 2009; Pope et al., 1991), success has been achieved in 12 species of nondomestic bovids (Morrow et al., 2009). The scimitar-horned oryx has been successfully bred by transcervical insemination totaling six births over two studies using frozen thawed semen (Garland et al., 1992). The use of general anesthesia is common for procedures as these, though the addax has received successful AI with manual restraint (Densmore et al., 1987). Estrus control was with a prostaglandin (PG) regimen with administration of equine chorionic gonadotropin (eCG) and prostaglandin. Also worth noting is that females used were halter broken and accustomed to handling. Frozen, thawed semen was used for insemination. Semen was collected from two males via electroejaculation. These animals were chemically restrained for collection (Densmore et al., 1987). Other species that have had success with AI include the blackbuck using fresh semen (Holt et al., 1988) and African buffalo using epididymal sperm collected postmortem (Friedman, 2000), proving the "Lazarus Effect" to be applicable and useful. There

has been no mention in each of these cases if the method of transcervical insemination requires rectal manipulation of the cervix.

Embryo Production

Procedures described and used with efficiency in domestics have been transferred to nondomestic ungulates. Transvaginal oocyte aspiration as well as post mortem collection has been performed with some success in nondomestic species, and the oocytes recovered are developmentally competent for use in in vitro maturation (IVM) and in vitro fertilization (IVF). There has been a mild, variable response to superovulation in ungulates including the scimitar-horned oryx, Arabian oryx and common eland (Durrant, 1983; Wirtu et al., 2009) as the ability to produce more than two ovulations under stimulation has been elusive to some. The use of IVF can help avoid such problems as fertility problems in some females, the difficulty of superovulation and amount of sperm needed to produce a pregnancy. Blastocyst stage embryos have been achieved in several antelope species using both fresh and cooled epididymal semen in IVF. Greater kudu (*Tragelaphus strepsiceros*) oocytes subjected to IVM/IVF with fresh and cooled epididymal semen had cleavage rates of 44% and 25%, respectively, with further development to morula and blastocyst stage was 18% and 26%. The impala (*Aepyceros melampus*) had low cleavage rates from oocytes collected via transvaginal aspiration (17%), but a high proportion of those that did cleave also developed to blastocyst stage (64%) (Loskutoff et al., 1995). Other species include the addax and bongo that have undergone in vitro techniques to produce blastocysts (Pope et al., 1991). Though these are promising achievements there are yet to be any live births achieved in antelope from in vitro procedures (IVM, IVF, IVC) (Wirtu et al., 2004).

There is some concern that the defects associated with domestic cattle and sheep IVF derived embryos could also be present in antelope should the techniques be utilized. Such defects include prenatal and postnatal fetal loss as well as large offspring syndrome which are

characteristic of in vitro derived embryos in domestic cattle. Conditions similar to these have been observed in other bovid species as the guar (*Bos gaurus*) from IVF derived embryos (Hammer et al., 2001).

Embryo Transfer

Many of the same superovulation techniques used with in vitro production are used for embryo transfer. In addition, embryo transfer (ET) is required for live birth after in vitro production. Challenges include the variations in cervix and uterus between species, most notably the duplex uterus and bifurcated cervix of the scimitar-horned oryx (Pope et al., 1991; Schiewe et al., 1991b). Other species with significant challenges include the giraffe (*Giraffa camelopardalis*) and okapi (*Okapia johnstoni*), giraffidae species, which present “near impenetrable cervices” (Loskutoff et al., 1995). The larger species of antelope (eland, bongo, oryx) allow for these challenges to be overcome more easily by rectal manipulation, which also aids in other techniques such as AI. Transcervical access has also been achieved in smaller antelope species [duiker (*Sylvicapra grimmia*), suni antelope (*Neotragus moschatus*), dik-dik (*Madoqua guentheri*)] from adaption of techniques used for sheep embryo recovery and transfer. In addition to adaption of ART techniques, the use of specialized chute systems (drop-chute, Tamer™, passive-crush) has contributed to the ability to successfully collect and transfer embryos. The use of these methods reduce the need for a surgical plane of anesthesia (Loskutoff et al., 1995; Wirtu et al., 2005), however, some sedation is usually used.

Nonsurgical embryo collection has been successfully performed in several species including the scimitar-horned oryx, bongo, eland, greater kudu, addax and sable antelope. Schiewe et al. (1991b) recovered 37 embryos from 10 successful flushes (n = 19 total attempts) in scimitar-horned oryx. Both embryos and unfertilized ova were recovered following superovulation of varying protocols in eland, bongo and greater kudu. More success that was

associated with these species was contributed to the bicornate uterus and single cervix exhibited as opposed to the bifurcated cervix of the scimitar-horned oryx (Schiewe et al., 1991b).

Live offspring have been produced in only four species, however [eland, bongo, suni antelope (*Neotragus moschatus zuluensis*) and scimitar-horned oryx] from in vivo collected embryos. The common eland has received success with embryo transfer, calving after intraspecific transfer of fresh and frozen embryos (Pope et al., 1991) and interspecific transfer has been attempted of eland embryo into a Holstein recipient (Dresser et al., 1982). The scimitar-horned oryx had success after intraspecific transfer of a morula stage embryo into a multiparous scimitar-horned oryx recipient, resulting in the birth of one healthy calf (see review by Pope and Loskutoff, 1999). Interspecies ET has also been attempted in the common eland as well as the scimitar-horned oryx, receiving bongo and Arabian oryx embryos, respectively. Although the scimitar-horned oryx failed to produce a pregnancy, the common eland was a success with the birth of a live bongo calf (Dresser et al., 1985). There are multiple applications for the use of interspecies embryo transfer, one such is the ability of a non-endangered surrogate to carry an endangered offspring (Kraemer, 1983). Possible explanations for failure of pregnancy in the scimitar-horned oryx may be the complex reproductive anatomy. It seems interesting to note however, that though the scimitar-horned oryx presents the most difficulty regarding cervical anatomy (bifurcated), the species has been successful where other species have not including artificial insemination, embryo recovery and embryo transfer resulting in live births.

Gamete and Embryo Cryopreservation

In addition to each of the techniques mentioned above, the use of genetic banking is a valuable resource for the future of conservation efforts and success. Cryopreservation has the potential to preserve gametes of endangered species until assisted reproductive technologies (ART) has been further refined in these species (Leibo and Songsasen, 2002). It has been

demonstrated that semen post-thaw remains viable in motility and ability to fertilize of various species (Schiewe et al., 1991a; Roth et al., 1999; Morrow et al., 2009). Efforts toward genome banking have begun, though further research is needed; optimum extenders for semen and sufficient cooling rates for oocytes and embryos need to be developed. Cryopreservation has proven to be successful in application to domestic species for the purpose of ART.

Cryopreservation has its greatest use in preserving and transporting cells for use in ART. This method also prevents removal of animals from the wild while still maintaining good genetic variation. Though cryopreservation has proven to be successful, the methods for freezing vary between species, cell types, even cell stage of embryos that result in the most viable outcome. Proof that cryopreservation leaves cells viable after freezing can be found in the healthy birth of many domestic species, but nondomestic successes are scarce. Oryx sperm is one such example, and as with most to all frozen-thawed sperm, motility and acrosome integrity is reduced but viability and fertilizability by oocyte binding is retained (Roth et al., 1998).

The future of cryopreservation is taking on more than just cells. Cryopreservation of tissue has been a goal but has not been realized for exotic species. However, while the attempt to freeze entire organs has not been successful, smaller tissue samples has. This success has a far reaching applicability for the endangered species; ovarian tissue slices were frozen, thawed and implanted back into the orthotopic site of sterilized ovaries of murine and ovine species (Newton and Illingworth, 1998). The insertion resulted in the birth of healthy offspring. While the initial implication of this study (Newton and Illingworth, 1998) was for human medicine, it can also be applied to endangered species conservation. Similar to genome and germplasm banking, slices of the ovarian cortex can successfully be cryopreserved. They can be banked and used as a resource for a variety of purposes including oocyte production in vitro or insertion of the tissue and oocyte production in vivo.

The ovarian cortex houses a large number of primordial follicles. These cells, being undifferentiated, reduce the likelihood of damage to them while freezing. Grafting of this tissue back into sterilized hosts should restore endocrine function and fertility. Fresh ovarian tissue and frozen-thawed tissue (frozen in DMSO to -196°C) were autografted to ovariectomized lambs at the ovarian pedicle on opposite sides (Newton and Illingworth, 1998). Two pregnancies resulted from mating ~ 3 months later. Two lambs were born, each from one of the tissue types; one from the fresh ovarian tissue, one from the frozen thawed tissue (Newton and Illingworth, 1998). Banking of ovarian tissue could also help to provide an alternate source of gametes for small captive breeding programs and help avoid the adverse effects of inbreeding. Follicles could be grown in vitro and used to introduce new genetics. In addition, should a captive breeding program fail, the ovarian tissue can be thawed and inserted into a closely related surrogate recipient for follicle growth (Newton and Illingworth, 1998). The use of cryopreservation holds potential for the future of conservation of antelope species though more work is needed to improve the efficiency of post-thaw viability of various species cells and embryos. Genome banking has begun in various institutions including The Wilds (Ohio) and the 'Frozen Zoo' at the ACRES facility.

Impact of Stress on Nondomestic Bovids

The effects of stress on nondomestic species can cause illness or even mortality. However, the concept of stress and its exact endocrine response is not fully understood, like much of the other physiological processes of these species (Hofer and East, 1998). It can be difficult to define stress per species as well as determine what specific event is stressful to the animal. What is determined is that stress has both a behavioral and physiological response, both manifesting according to the individual's assessment of the stressor (Axelrod and Reisine, 1984). While behavioral responses to stress may be presented in various ways as aggression, flight, or other coping, the physiological response is a product of the hypothalamic-pituitary-

adrenocortex axis (Moberg, 1987). The basic concept is that a stressor stimulates the adrenals and a subsequent secretion of catecholamines or glucocorticoids enters the system. One of the disadvantages of working with nondomestic animals is their fractious nature and susceptibility to 'stress'. Some species are considered to be more fractious than others, for example, the eland is thought to be considerably calm in relation to other antelope species (Hansen et al., 1985).

A condition known as capture myopathy has been described in nondomestic ungulate species. The symptoms associated with capture myopathy are depression, stiffness, weakness, recumbency and can even include paralysis (Chalmers and Barrett, 1977). Capture myopathy can also exhibit itself as shock and muscle damage due to lactic acidosis and increased body temperature resulting in renal failure, an inability to regulate homeostasis and sudden death (Spraker, 1993). It can also be difficult to determine if a significant amount of stress is being experienced by the animals or if the stressor is "bad". The adrenal response is the same and does not distinguish between adverse or beneficial events such as being attacked by a predator or copulation (Colborn et al., 1991). Short periods of acute stress are considered to be non harmful or even good, chronic or prolonged stress can interrupt other physiological systems as immune response and reproductive responses (Munck et al., 1984; Liptrap, 1993).

The use of long acting neuroleptics has been shown to lessen the effects of adverse stressors and recurring handling for procedures. One such drug, Zuclopenthixol acetate, was used in wapiti, a North American elk species (Read et al., 2000). The initial formation and use of the neuroleptic drugs was for human anxiety. In animals, they have been shown to reduce aggression and evoke an indifference to new stimuli and general calming (Ebedes and Raath, 1999). The drug (Zuclopenthixol acetate) was in effect for up to 4 days after initial day of administration. When compared to control animals, those treated with the drug maintained lower body temperatures, lower cortisol levels, less hemoconcentration, lower blood lactate levels and were less metabolically acidotic. Creatine phosphate levels were lower in treatment animals as

well. It is important to take multiple parameters into account when assessing the stress level of an animal. The 'classic' indicator of stress is cortisol, but other indicators viewed in combination with cortisol would give a better image of the animals' situation. Each of these symptoms can be attributed to physiological processes that occur when a prolonged stress is experienced. The most common behavioral reaction to stress is pacing and increased activity. This leads to increased heat production and oxygen consumption. These lead to anaerobic metabolism, resulting in metabolic acidosis and, if uncontrolled, shock and death.

An increase in hemoconcentration can be attributed to activation of the adrenals releasing catecholamines causing a contraction of the spleen and bulk release of erythrocytes (Read et al., 2000). Knowing the physiological processes that occur will help determine what parameter to evaluate when assessing stress. The most simple would be to monitor rectal temperature, but a normal resting temperature would need to be established. The same establishment of basal levels would be necessary for each of the parameters mentioned. But in combination and with ranges defined for various species they can be an excellent way to monitor the stress level of the animal. Further research and work is still needed to define these ranges and determine species specific values. The use of long acting neuroleptics could be beneficial for aid in studies of ungulates in the future. However, it would need to be determined if the drugs used would affect any of the processes of reproduction, such as circulating hormone levels or other physiological processes.

On the focus of reproduction, a single episode of restraint and relocation may disrupt ovulation (Moberg, 1987). Plasma glucocorticoid levels can aid in determining if capture by either manual or chemical restraint is stressful as plasma cortisol levels rose after both methods of restraint. It was determined however, that chemical restraint was less stressful (i.e. return to basal cortisol levels) than manual restraint in a variety of wildlife species (Morton et al., 1995). Cortisol metabolites have been measured in feces successfully and can be used as a

convenient and noninvasive method of cortisol evaluation (Mostl and Palme, 2002). Cortisol and cortisol metabolites were determined to be useful indicators of stress as plasma cortisol levels did fluctuate in response to either restraint method (Morton et al., 1995). The drugs used in chemical restraint are often a combination of xylazine and opiate agonists such as ketamine or etorphine. When chemically restraining nondomestic ungulates precautions must be taken similar to those taken with domestic ruminants to avoid conditions such as ataxia, bradycardia and bloating. In the addax, recumbency was achieved in 5 minutes following etorphine and detomidine combination and 11 minutes following medetomidine and ketamine administration. Both drug combinations were found to be suitable for initial sedation and recumbency for clinical procedures including lameness evaluation and digit amputation. Supplemental agents can be administered if a deeper or prolonged light plane of anesthesia is required for manipulations (Portas et al., 2003).

There is discord however, in the opinion of the better handling system for nondomestic ungulates. Morton et al. (1995) found that chemical restraint of various nondomestic ungulates returned to a basal plasma cortisol level faster than in manually restrained. However, Atkinson et al. (1999) found that if a suitable restraint system, such as a Tamer™, were used for consecutive days then a habituation effect was evident and plasma cortisol levels decreased. Also, larger nondomestic ungulates such as the eland can be habituated to a regular regime without significant detriment to health or death (Wirtu et al., 2005). When restrained repeatedly for minimal manipulation, blood glucose levels rose, but creatine and hematocrit levels remained basal when compared to animals only being restrained (Wirtu et al., 2005). Size of the animal can determine which method would be more efficacious, as well as intent for the restraint of the animal.

For restraint in a non captive setting or for veterinary procedures that require sedation or anesthesia, chemical methods would be better for the one time episode. On the other hand,

restraint on a captive setting for consecutive episodes of restraint, manual restraint with the proper housing and restraint system would be preferred. For some techniques that require the animal to be in a dorsal or standing position, like ultrasound guided oocyte aspiration, nonsurgical embryo flush and artificial insemination a combination of light sedation and use of a chute system would be also be a choice method (Wirtu et al., 2005). The most stressful episode seems to be travel for these species, and results most often in injury or death, even when sedated for travel (Morton et al., 1995). Depending on the intended procedure for the eland, any one of the described methods would be acceptable for proper restraint of large nondomestic ungulates, with the exception of travel.

CHAPTER III

CHARACTERIZATION OF THE COMMON ELAND (*TAUROTRAGUS ORYX*) ESTROUS CYCLE THROUGH OBSERVATION OF BEHAVIOR, ULTRASONOGRAPHY AND BLOOD SAMPLING

Introduction

Three species of the antelope tribe tragelaphini (spiral horned) are considered endangered by the IUCN (2002), including the eastern bongo and mountain nyala. While efforts have been made to improve the numbers of these endangered species, relatively little is known about their estrous cycles. One method of advancing the understanding of nondomestic species reproduction is the adaption of techniques commonly used in domestic cattle to nondomestic ungulates. The common eland lends itself to this approach due to the large frame of the species and its relatively calm nature. The adaption of such assisted techniques as artificial insemination, embryo transfer and in vitro fertilization are all options to apply to nondomestic reproduction, some of which have been achieved, although with low efficiency (Dresser et al., 1984; Pope et al., 1991; Luskutoff et al., 1990; Schiewe et al., 1991a, b; Morrow et al., 2009).

In addition to efficiency of reproduction, the ability to transport genetics to maintain genetic viability within captive populations is also a concern and point of interest (Luskutoff et al., 1995; Wildt and Wemmer, 1999). Semen collection has been performed with success and the transport of this, as opposed to the transport of an animal, is much preferred. Knowing and understanding the characteristics of the nondomestic ungulate estrus would enable the most efficient use of semen for artificial insemination. Along similar lines, the understanding of these characteristics would also enable the most efficient use of other techniques used in domestic cattle reproduction. However, before any of these techniques can be utilized by conservationists, a detailed characterization of the estrous cycle needs to be completed.

The purpose of this study was to characterize the common eland estrous cycle. Specific aims include determining time to ovulation after two estrus synchronization methods, follicle size at ovulation and behavior around the time of estrus.

Both the nature of these animals and the number of animals available to use were points of consideration when designing this study. Eight females were available for research for this study at the Audubon Center for Research of Endangered Species (ACRES). There are other eland on the property but they were not allowed for use in research. Housing changes were made prior to the onset of the study to remove various factors that could have influenced the study. Due to the amount of stress that this study did entail, a crossover study period was used to allow a rest period for the experimental animals and to expose all study females to each synchronization regimen.

Materials

Experimental Animals

The animals used in this study were common eland antelope (*Taurotragus oryx*) housed as a single bachelorette group containing 11 members. The additional 3 animals were not used either due to age or overt fractious nature. The study utilized eight adult female individuals that ranged in age from 4 to 13 years and weights ranged from 350 to 377 kg. Table 3.1 lists the animal ages, weights, body condition score, and subjective assessment of temperament and associated score. Animal body condition scores (1 = lean to 9 = obese), subjective assessment of temperament and associated temperament scores (T-scores with 1 = calm and 4 = fractious) were assigned to each animal prior to treatment. These T-scores give an indication of the social dominance groupings within the overall bachelorette herd.

The location of the experiment was at ACRES in New Orleans, Louisiana. The climate is considered semi-tropical in this region of the northern hemisphere. The study was conducted

Table 3.1 The ages, weights, body condition score (BCS, 1 - 9), relative temperaments and associated scores (1 – 4) of each eland female at the start of the study.

Animal ID	Age	Weight	BCS (1 – 9)	Temperament	Score (1 – 4)
123	12 years*	345 kg	5	calm	1
129	12 years*	377 kg	6	calm, food motivated	1
139	10 years	349 kg	5	aggressive	4
259	4 years	350 kg *	5	fractious	3
140	10 years	361 kg	5	calm	2
159	11 years	350 kg *	5	fractious	3
161	13 years	350 kg *	4	fractious	3
258	4 years	350 kg *	5	fractious	3

Figures indicated with an (*) are estimates due either to uncertainty of age or weight. Those with estimated weights were too fractious to be weighed standing.

from September 2007 to March 2008. This season (late fall and winter) was selected for the cooler temperatures to reduce the amount of heat stress that would be experienced as opposed to the much higher temperatures of the summer months in southern Louisiana. Although the barn and chute are enclosed and air conditioned, it was thought that the stress that would be experienced would be better tolerated with cooler ambient temperatures.

Animals were distinguished by individual markings, ear tags and reflective tape placed around the horns in individual patterns. A male common eland is normally housed adjacent to the bachelorette enclosure, however, he was moved to another enclosure with other eland females on the ACRES compound prior to onset of the study. Due to the distance (~0.8 km) and presence of other animal enclosures between the herds, the male had little, if any, communication (i.e., visual, audio) with the study females. All females were in good body condition at the start of the study. They were fed pelleted feed (Mazuri® CU ADF-16, PMI Nutrition International, Brentwood, MO) as a single herd once or twice per day. The concentrate contains 17% crude protein and 3% crude fat minimally and 15% crude fiber and 14% acid detergent fiber maximally. In addition, they received a bail of alfalfa hay once per day. Periodically the females were fed “treats” (bananas) that would become crucial for regimen administration. Water and salt lick were available *ad libitum* in various places around the enclosure.

The previous breeding histories of these females are not known, but none of the females used in this study had calved while housed at ACRES. All females in the bachelorette herd are housed in a large open yard (45 m x 56 m) surrounded by a chain link fence ~4 meters tall. In the center of the yard is a concrete feeding pad (7.6 m x 2.5 m) that is roofed and also surrounded by chain link fence with remotely operated sliding fence doors. The feeding pad is connected to a barn with two adjacent rooms that lead to the Tamer™ (Fauna Research Inc., Red Hook, NY) hydraulic chute. The Tamer™ chute is designed to squeeze and lift the animal

for manual restraint. It allows access, via side doors, to the neck and leg regions on both sides of the chute; these openings allowed for safe blood sample collection. The final room of the barn also opens to a small yard adjacent to the larger enclosure, also connected. This enclosure design is illustrated in Figure 3.1. The interior of the barn and chute was air conditioned for veterinary and other procedures performed during warmer months.

Routine veterinary care included biannual worming preventative administration. Agents used were: fenbendazole (Panacur[®], DPT Laboratories, San Antonio, TX), pyrantel pamoate (Strongid[®]-T, Pfizer Animal Health, Exton, PA) and ivermectin (Ivomec[®], Merial Ltd, Iselin, NJ) and rotated between events. Selenium and Vitamin E was also administered biannually. Annual vaccinations included rabies (RabvacTM-3, Fort Dodge Animal Health, Fort Dodge, IA), Clostridia (Fortress[®]-7, Pfizer Animal Health, Exton, Pennsylvania) and Leptospira (Leptoferm-5[®], Pfizer Animal Health). Fecal samples are examined four times per year and treatments are given as needed. The ACRES Institutional Animal Care and Use Committee approved all procedures performed. A staff veterinarian was available to examine the animals as needed throughout the project. One advantage to the study was that these females were used previously in experimental procedures utilizing this enclosure and chute system (Wirtu et al., 2004, 2009).

Treatment Periods

A crossover animal observation period was used in this study. The study females were separated into two groups of four ($n = 8$) and received two regimens each. The regimens were estrus induction agents that were administered to each group. Figure 3.2 illustrates the timeline of regimen administration. Regimen 1, a commercially available prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Lutalyse[®], Upjohn Co., Pfizer Inc.) was given on day 0 and day 11. Regimen 2, a commercially available altrenogest (Regumate[®], SmartPak Equine LLC, Plymouth, MA), administered orally from day 0 to day 7 and $PGF_{2\alpha}$ given on day 7. Figure 3.3 illustrates the

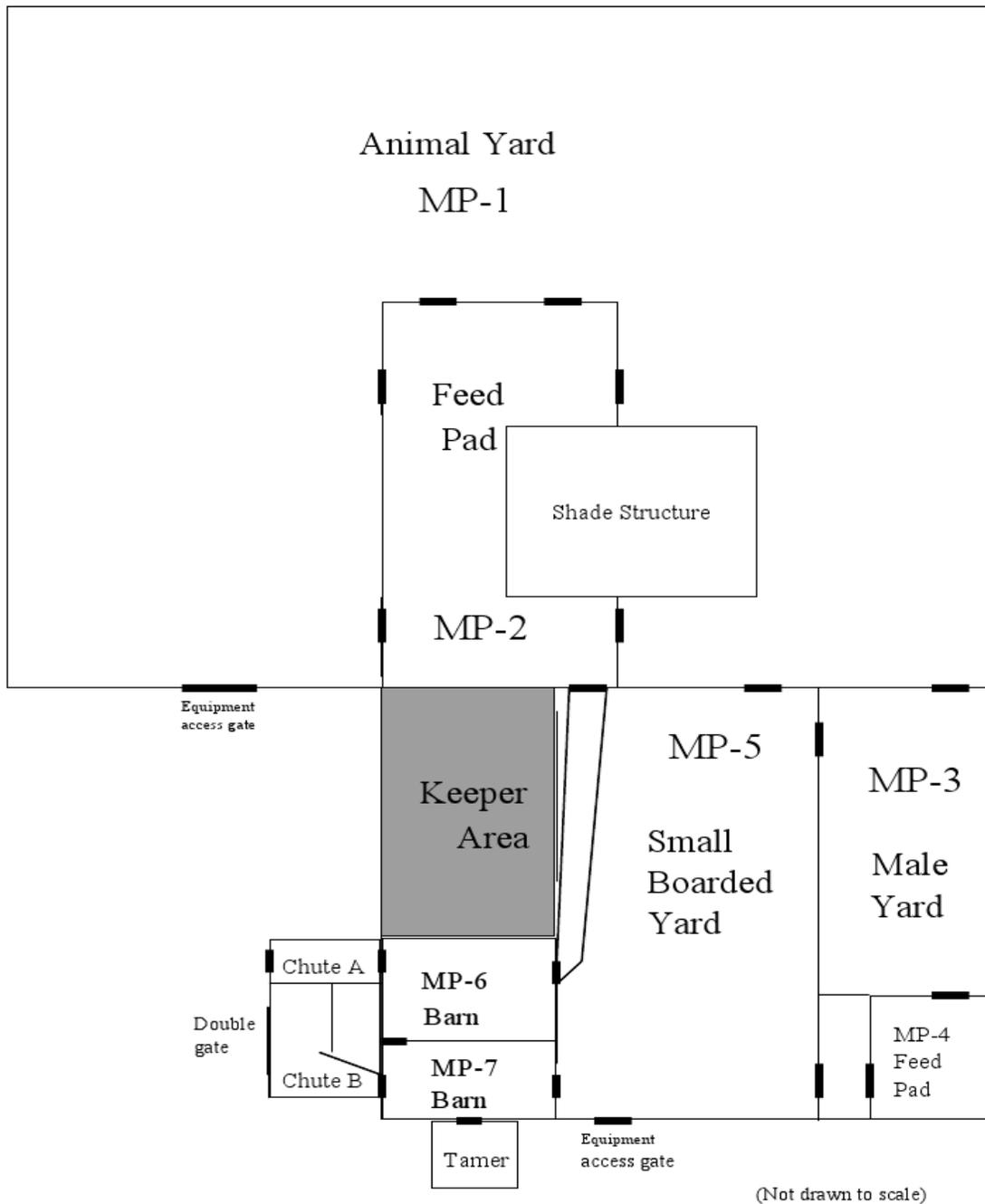


Figure 3.1 Schematic for the enclosure of the female eland yard. Main holding area and yard (MP-1), feeding area with shade (MP-2), temporary holding area with shade (MP-5), holding and feeding areas for a male eland (male was removed for project) (MP-3, MP-4), keeper area, barns (MP-6, MP-7) leading to hydraulic chute (Tamer). Gates and doorways remotely operated from keeper area. Barn (MP-7) is equipped with remotely operable push-wall toward Tamer. Schematic is not drawn to scale. (Courtesy of E. Sarrat, ACRES)

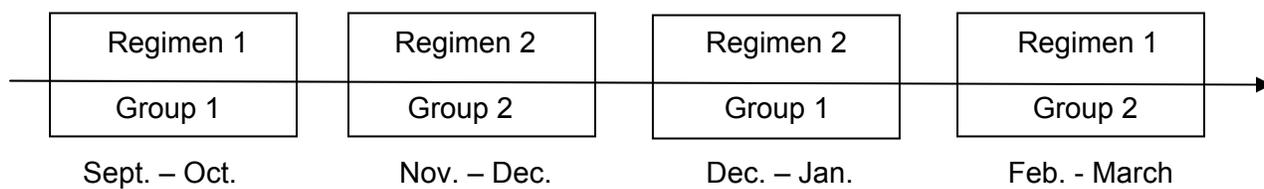
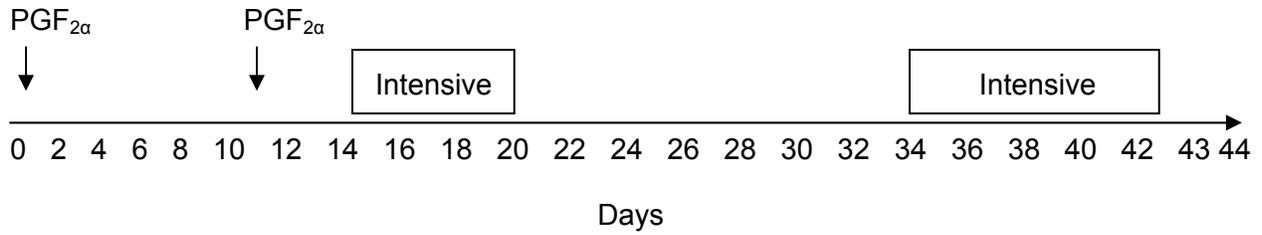


Figure 3.2 The progression of regimens in a crossover study period and time of year they were completed. Each regimen includes estrus induction, as well as, subsequent natural estrus.

Regimen 1



Regimen 2

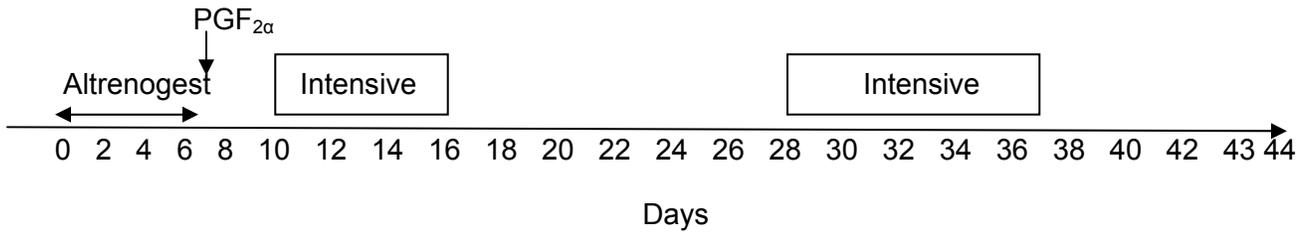


Figure. 3.3 The regimen schedules for estrus induction (Regimen 1 or 2). Intensive sessions were performed after induction until time of ovulation. Approximately 21 days after ovulation of induced estrus, a second intensive session was performed until ovulation during a natural cycle.

induction agent administration timeline. Once each group received one regime, they subsequently received the other with a recess from observation between regimens: Group 1 received Regimen 1, then Regimen 2; Group 2 received Regimen 2, then Regimen 1. Each occurred consecutively over four time periods.

PGF_{2α} (25 mg) was given i.m. in regimens 1 and 2. The altrenogest was orally fed (0.22 % in oil; 2.2 mg, 5mL per head per day). Altrenogest has been successfully used to induce estrus in common eland in previous studies at the ACRES compound (Wirtu et al., 2009). To ensure that the females received the full dose of altrenogest the solution was inserted into bananas, between the skin and the meat of the fruit and fed to each animal through the fence. The utilization of 'treats' (bananas) for administration of the altrenogest was crucial to successfully complete Regimen 2.

Methods

Detecting Estrus

Initially cameras and recording monitors were arranged around the perimeter of the enclosure to record behavior surrounding estrus. Due to blind areas from camera placement, adhesive scratch off heat detector patches (Estroprotect™, Select Genetics, Washington, PA) were then applied to the tail head of animals. The patches however, came off on occasion and the amount of mounting became additive, making determining when mounts were performed difficult. Also, some objects in the enclosure, such as trees and fencing, were low enough to scratch the patches. In addition, we would later see that false negatives were possible as the mount duration is not long and patches were not scratched. As a remedy to these situations we applied the computer-based HeatWatch® (CowChips, LLC, Manalapan, NJ) system to the animals and we could quantitatively count the number of mounts performed and received by the females. The transponders of the HeatWatch® system were able to record female mounts of

less than 2 seconds, making us more confident in the accuracy of readings from this approach.

One female not used in this study was administered 4 testosterone implants (200 mg; Synovex-H, Fort Dodge Animal Health, IA) subcutaneously in the left ear. This androgenized female exhibited mounting behavior on those females coming into estrus and was used as a behavioral indicator of estrus. Females standing to be mounted would be considered in estrus.

Ultrasonography

When females under observation were expected to be in estrus (~ 3 days after final PGF_{2α} injection) each female was evaluated via ultrasound. Ultrasound sessions were performed every 24 hours until a large follicle disappeared from the ovary. Then, 19 to 20 days after suspected ovulation the females underwent ultrasound sessions every 24 hours while the female was predicted to be in natural estrus until the disappearance of a large follicle or discontinuation of follicular growth. The number of follicles on each ovary was recorded in addition to ovary size for each female. An Aloka ultrasound unit (SSD-500V model, Corometrics Medical Systems, Inc., Wallingford, CT) with a 7.5 mHz probe fitted with a rigid piece of plastic was used. The rigid piece was to aid in maneuvering while in the rectum as the space was small and the least amount of stress and insult to the rectal wall was our goal. Each ultrasound session lasted 30 to 45 minutes per animal and was performed by the same technician to maintain consistency. Upon disappearance of a large follicle ovulation was suspected. Ovulations were later confirmed in females with circulating luteal phase progesterone levels. Corpora lutea were not visualized in order to lessen the amount of time each female was intensely observed and reduce the amount of stress. Each ultrasound session was recorded to a CD through a DVD recorder to document each animal and for later evaluation.

Blood Sampling

Blood samples were taken 3x per week from start of synchronization administration, when the group under observation was expected to be in estrus sampling increased to every 12 hours. Once the female was assumed to have ovulated (disappearance of a large pre-ovulatory follicle) or follicular growth discontinued sampling resumed to every 3 days. Then, 19 to 20 days after suspected ovulation, sampling again increased to every 12 hours while the females were in peri-estrus.

Samples were taken by veinipuncture of the jugular vein while the animal was restrained in the Tamer[®] chute. Blood was collected via 18 gauge Vacuatener[®] needles into heparinized, 5mL glass tubes. Samples were centrifuged and plasma aliquots (in duplicate) were frozen for storage at LSU at 4°C until hormone analysis via previously validated radioimmuno assay (RIA) and enzyme linked assay (EIA). RIA assays were run at Louisiana State University and EIA assays were run at White Oak conservation center (Yulee, FL). Animals were in standing position, squeezed and lifted with the chute for both blood collection and ultrasonographic sessions.

Hormone Assays

Commercial radioimmuno assay kits were purchased from DSL (Diagnostic Systems Laboratories, Webster, TX) for analysis of plasma samples for assay of hormones progesterone, estradiol-17 β and luteinizing hormone (LH) was analyzed with an “in house” assay (Thompson et al., 1983) thus, no kit was purchased. The individual protocols for each hormone assay and each female are provided in Appendix A.

Samples were frozen for storage and thawed in a warm water bath (37°C) for each assay. The common eland is a ruminant species thus samples had to be extracted with acetone (progesterone), or diethyl ether (estradiol-17 β) before proceeding to the addition of antibodies.

The progesterone (P_4) assay utilized a double antibody system of rabbit-anti progesterone and goat-anti rabbit antibodies. The estradiol- 17β assay also used a double antibody system of rabbit anti-estradiol and goat anti-rabbit antibodies. LH assays were not extracted as it is a protein assay, the antibodies used for this were anti-LH and anti-rabbit gamma globulin. Assay validation for progesterone, estradiol and LH as described Thompson et al. (1983).

The radio-labeled hormones used in these assays were I^{125} progesterone, estradiol- 17β and LH. Assay was conducted as previously described by Thompson et al. (1983). Briefly, steroid hormone assays (progesterone and estradiol- 17β) were competitive binding assays in which the labeled hormone and first antibodies (rabbit anti-progesterone, estradiol) were added in a single step. After a period of incubation at 4°C the second antibody was added (goat anti-rabbit). After the final incubation at 4°C , samples were washed and centrifuged twice, after decanting the second wash tubes were loaded into the gamma counter for value readings. LH was not a competitive assay thus antibodies were not added in union. Inter- and intra-assay variation was 5% and 8% for P_4 , 7% and 10% for E_2 , and 6% and 9% for LH, respectively.

Results

Animal Behavior

Homosexual mounting behavior was observed between females and females standing to be mounted were considered in estrus. Other behavior indicating estrus was a female flehmen response, repeated head butting and close following of a particular female. Mounting behavior by the androgenized female was observed and a total of 52 mounts were recorded for all females. Mounts were numerically evenly distributed throughout three quarters of the 24-hour period: 12 am to 6 am, 29% (early morning), 12 pm to 6 pm, 29% (early afternoon) and 6 pm to 12 am, 33% (late afternoon). The fewest mounts (10%) were recorded during the 6 am to 12 pm, 10% quadrant (late morning) (Figure 3.4). Chi square analysis ($p= 0.886$) confirmed that

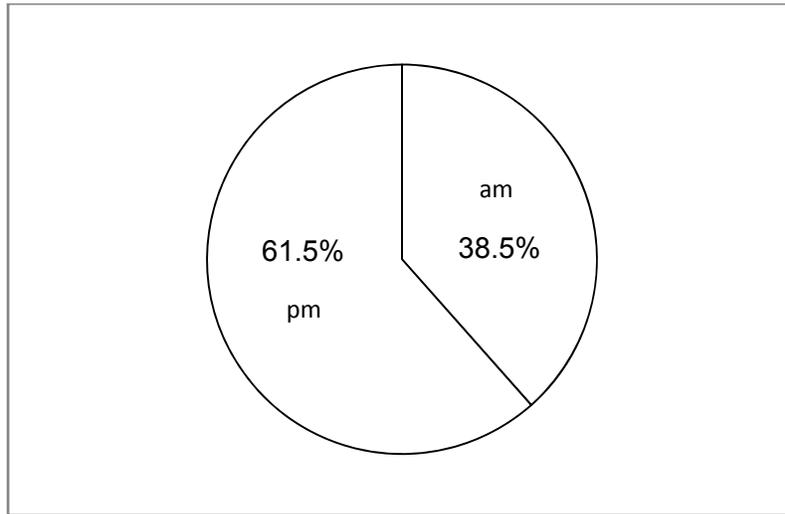


Figure 3.4.1 The number of mounts during halves of the day. The majority of mounts were recorded during the hours that fall under pm (61.5%) during the day and fewer mounts were recorded for the am hours (38.5%).

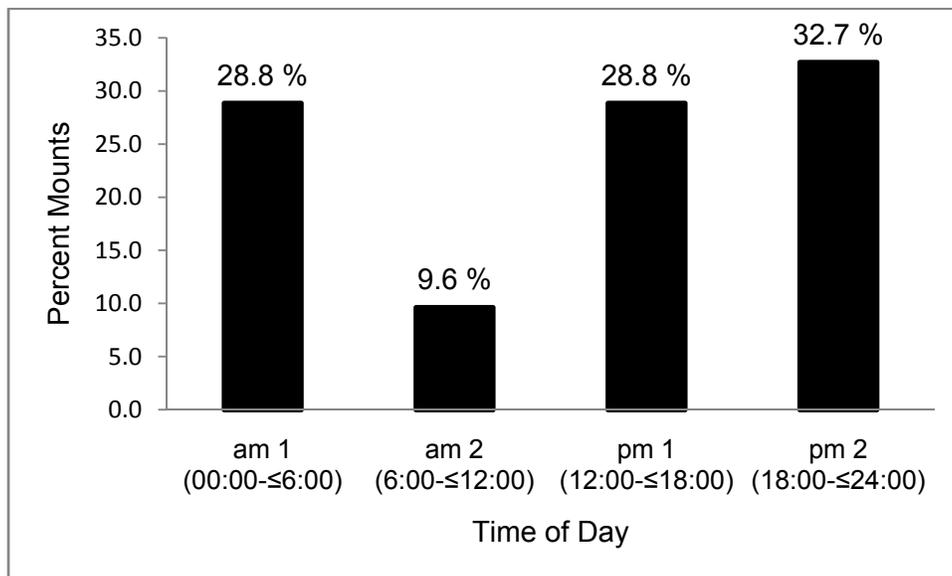


Figure 3.4.2 The percentage of mounts (≤ 2 seconds) recorded during quadrants of the day. The fewest mounts were recorded during the late morning (0600 – 1200).

there was no association between time of day and number of mounts. When the 24 hour day is split into 12- hour segments, the majority of mounts, 61.5%, were recorded during pm hours while 38.5% were recorded during am hours.

Each mount duration was 2 seconds or less and the most mounts received by a single female were 20. Estrus lasted from 2.1 to 29.0 hours. For females that were suspected to have ovulated, the average time from initial mount to ovulation was 1.6 days with a range of 0 to 5 days. Though it was not considered as a part of this study, swelling of the vulva was observed during intensive sessions (estrus), Figure B1 (Appendix B).

Ultrasonography

Ultrasound during estrus showed that visualization of the ovarian structures and rectal manipulation of the common eland reproductive tract is possible without sedation. Follicle size and number could be determined as well without sedation. Seven ovulations of 30 possible (23.3%) were detected by the criteria of disappearance of a large follicle (7 to 10 mm) after consecutive viewing every 24 hours. When compared to with the mounting data, standing estrus was on average 1.6 days (range: 0 to 5 days) prior to detectable ovulation via ultrasound. The largest follicle size consistently recorded was 10 mm. Examples of images recorded are given in Figure 3.5. These findings were later confirmed with circulating progesterone concentrations. Five of 15 ovulations were detected (33.3%) after induction of estrus (Regimen 1 or Regimen 2: 3 and 2 ovulations, respectively). One animal ovulated during both the induced cycle and the subsequent natural cycle (#140) following Regimen 1, each ovulation occurred on alternating ovaries. In addition, the ovulations detected occurred as frequently on the left (3: 42.8%) as on the right (4: 57.1%) ovary. Interval from final PGF_{2α} injection to ovulation was 69 hours on average, with a range of 58 to 82 hours. This was determined by averaging the shortest and longest time to possible ovulation and adding the shortest possible amount of time for each ovulation.

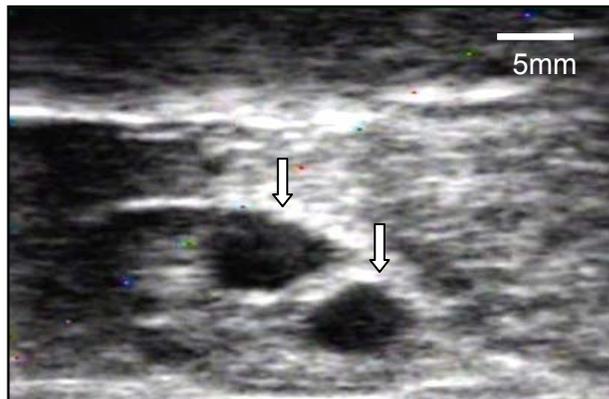
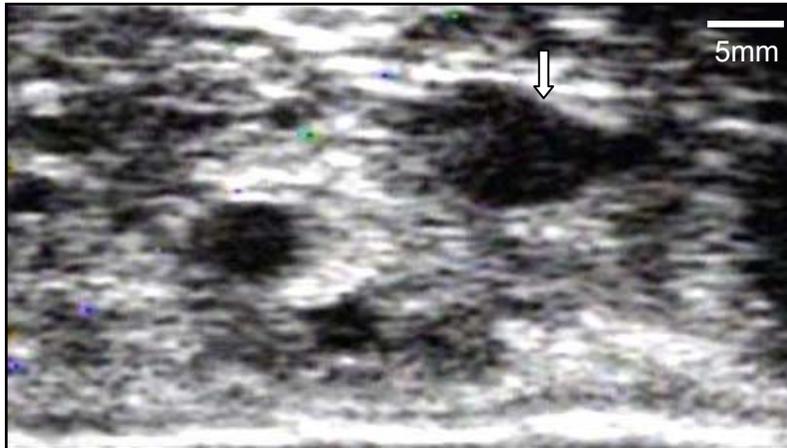


Figure 3.5 Images recorded from ultrasound sessions. The top image shows one large follicle, ~10 mm with other smaller follicles. The bottom shows two medium- size follicles (~6 mm) with arrows indicating location of follicles. The dark circular spaces indicate the presence of fluid within a more dense ovarian structure.

Inter-ovulation (\pm SE) interval was determined by the number of days between growth of a large follicle and was determined to be 21 ± 1.6 days with a range of 18 to 25 days. Of suspected ovulations, no more than two follicles were present at time of ovulation and the ovulating follicle was clearly larger. All animals developed both small (≤ 3 mm) and medium-size (4 to 6 mm, respectively) follicles. The number of females that had follicles at each size interval and under which regimen the ovulations are shown in Table 3.2.

Hormone Profiles

Blood samples that were taken 3x per week between estruses and every 12 hours during estrus were used to generate plasma hormone profiles from induced estrus to natural estrus. The profiles show that common eland hormones can be assayed for and followed over time. Progesterone levels remained low (≤ 2 ng/mL) for RIA assays, but trends were similar with EIA assays, confirming the RIA assays. As EIA trends were similar to RIA assays no further investigation was conducted through EIA. Estrogen and LH were only assayed via RIA.

Progesterone was the primary hormone that was investigated to confirm suspected ovulations from ultrasound. Progesterone declined after final $\text{PGF}_{2\alpha}$ injection in 11 of 15 (73%) possible estrus inductions (female # 123 was injured at the start of Regimen 2). RIA values ranged from 0.01 ng/mL to 2 ng/mL for progesterone. EIA values ranged most frequently from 0.05 ng/mL to 10 ng/mL. The combined RIA profiles (progesterone, estrogen, LH) of the females and regimens that were suspected to have ovulated are presented in Figures (3.6 - 3.11). Further supplemental figures of hormone profiles are provided in Appendix C for all animals during the study. EIA assays were only run for progesterone analysis to confirm RIA trends and ovulations suspected during ultrasound. Estrogen levels ranged from 0 to 2 pg/mL most frequently. LH levels remained below 3 ng/mL with the exception of two occasions, where on both occasions LH concentrations rose above 7 ng/mL. These apparent LH peaks occurred

Table 3.2 The number of follicles that appeared during each regimen induction and subsequent natural estrus observations (bottom portion of table 3.2) during intensive sessions.

Regimen Group	Number	No. observed	No. w/ ≤ 3 mm	No. w/ 4 – 6 mm	No. w/ 7 - 10 mm	Proposed ovulation
Regimen 1 d 13 – 15	4	8	8/8 (100%)	8/8 (100%)	5/8 (62.5%)	2/8 (25%)
Regimen 2 d 9 – 11	4(3 [*])	7	6/7 (85.7%)	6/7 (85.7%)	4/7 (57%)	3/7 (42.8%)
Regimen 1 d 33 – 40	4	8	8/8 (100%)	8/8 (100%)	4/8 (50%)	1/8 (14.3%)
Regimen 2 d 29 – 36	4(3 [*])	7 [*]	7/7 (100%)	7/7 (100%)	4/7 (57.1%)	1/7 (12.5%)

The (*) indicates that animal #123 was injured during Regimen 2 and was removed from the project.

Female #129 Regimen 1

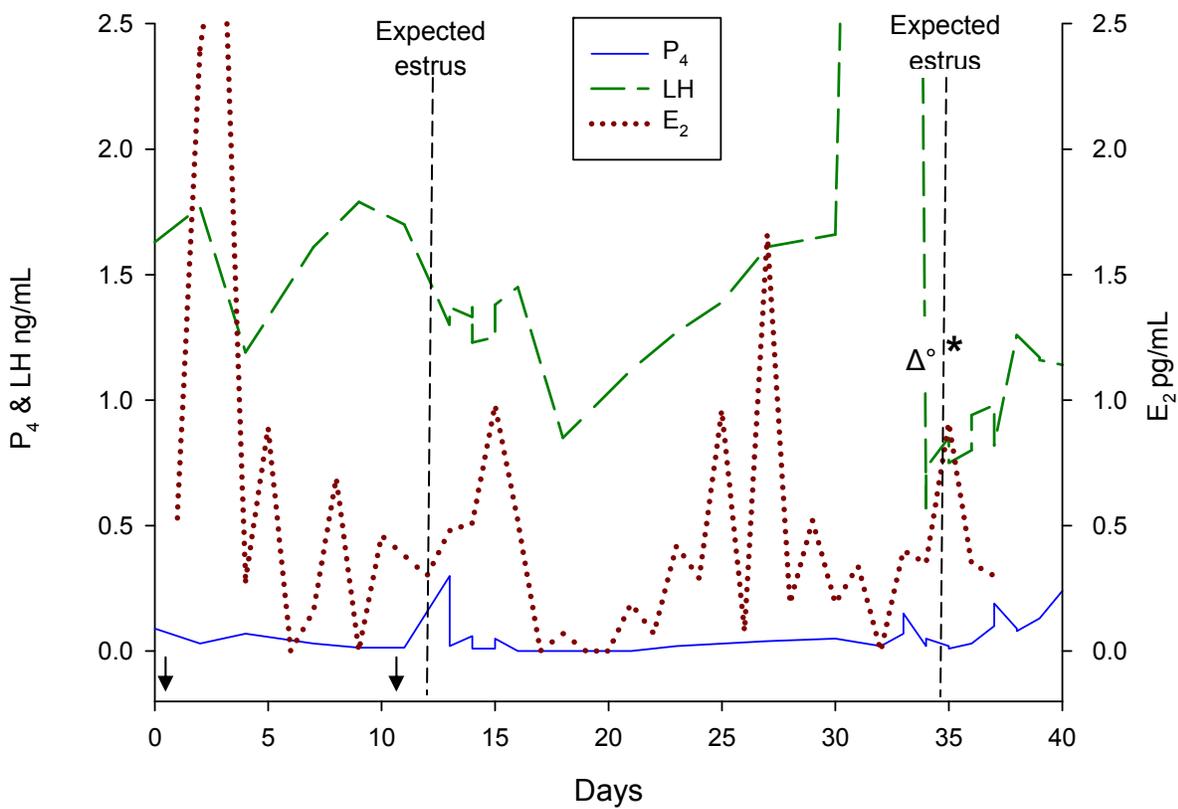


Figure 3.6 The combined hormone profiles for progesterone, estrogen and LH for animal #129 during Regimen 1 (Lutalyse protocol). We suspect this to be an LH peak prior to ovulation (*) (day 35; 7 mm) during the natural cycle, standing estrus was day 34 (Δ°) of the sampling period. Arrows indicate PGF_{2α} administration on days 0 and 11.

Female #129 Regimen 1

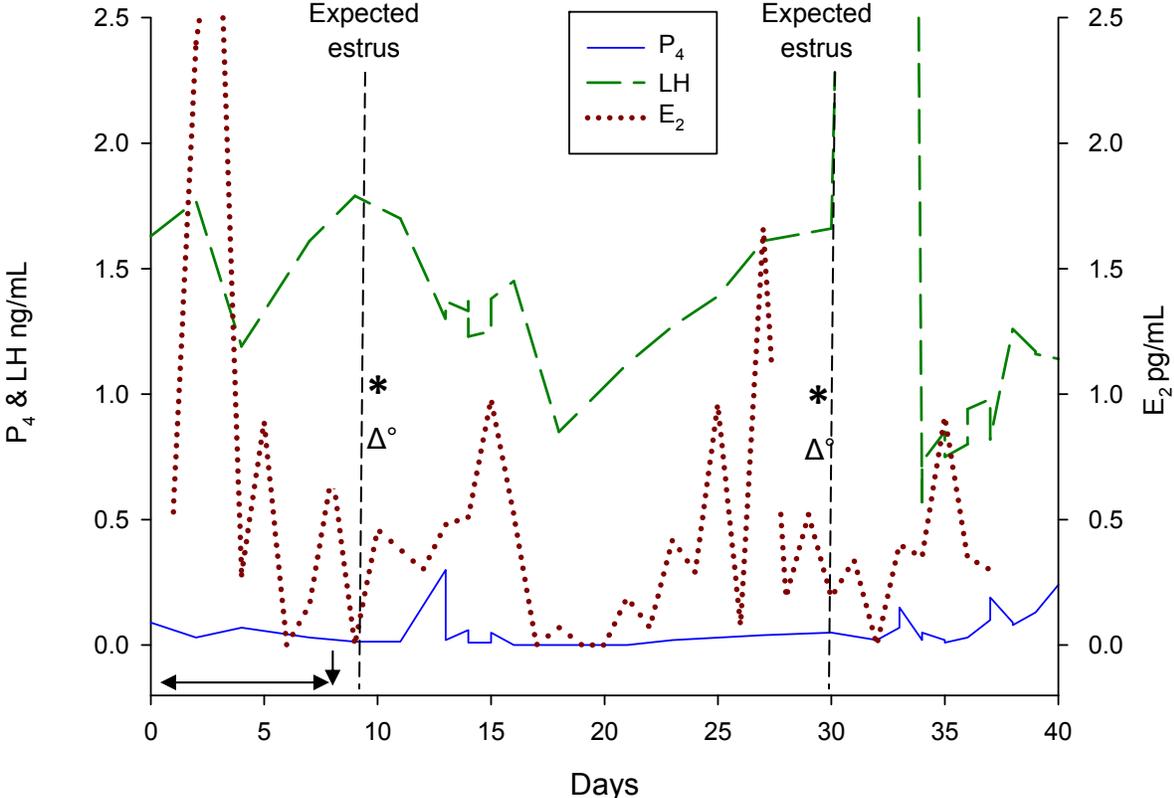


Figure 3.7 The combined hormone profiles for progesterone, estrogen and LH for animal #140 during Regimen 2 (altrenogest protocol). This particular animal was suspected to have ovulated (*) during both the induced estrus (day 9; 7 mm) and subsequent natural cycle (day 29; 10 mm) though estrogen does not fluctuate greatly. Standing estrus (Δ°) was observed at time of ovulation. Vertical arrow indicates PGF_{2 α} administration, horizontal indicates altrenogest administration.

Female #161 Regimen 2

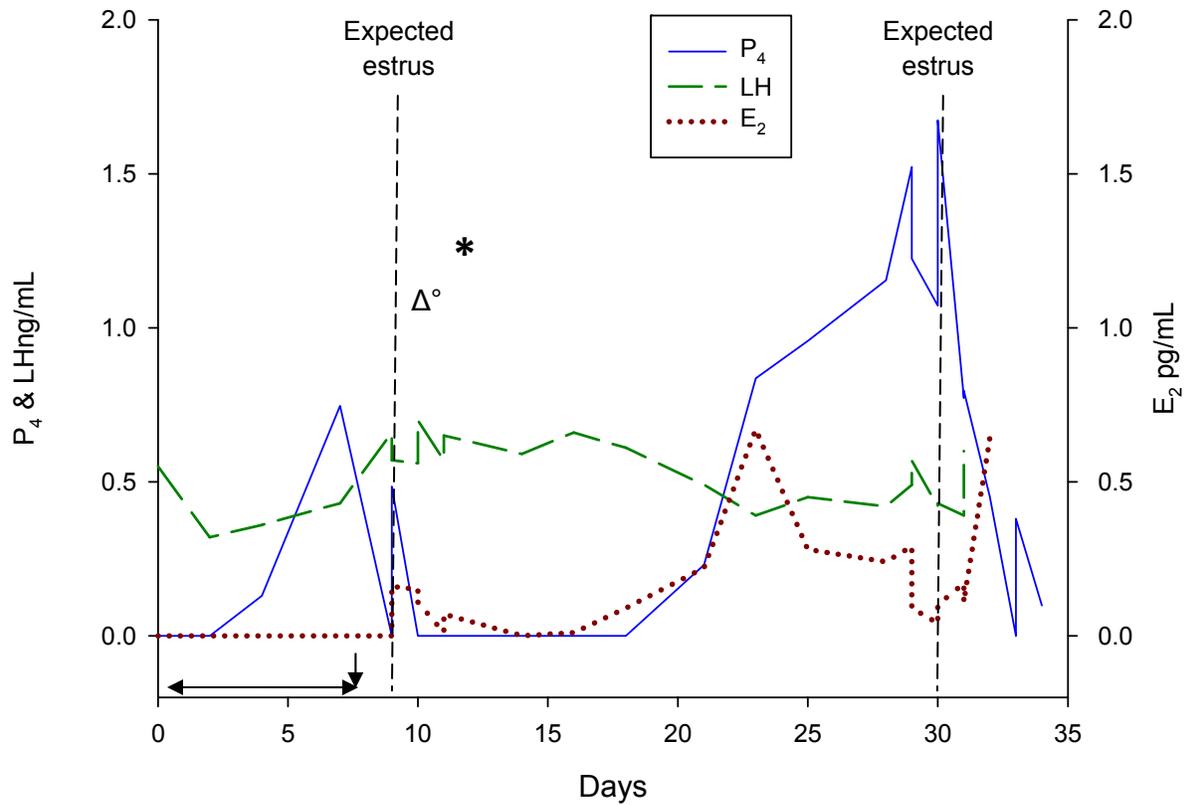


Figure 3.8 The combined hormone profiles for progesterone, estrogen and LH for animal #161 during Regimen 2 (altrenogest protocol). Ovulation (*) was suspected to have occurred following induction (day 11; 8 mm) and standing estrus (Δ°) was observed on day 10. Vertical arrow indicates PGF_{2 α} administration on day 7, horizontal indicates altrenogest administration days 0 through 7.

Female #259 Regimen 2

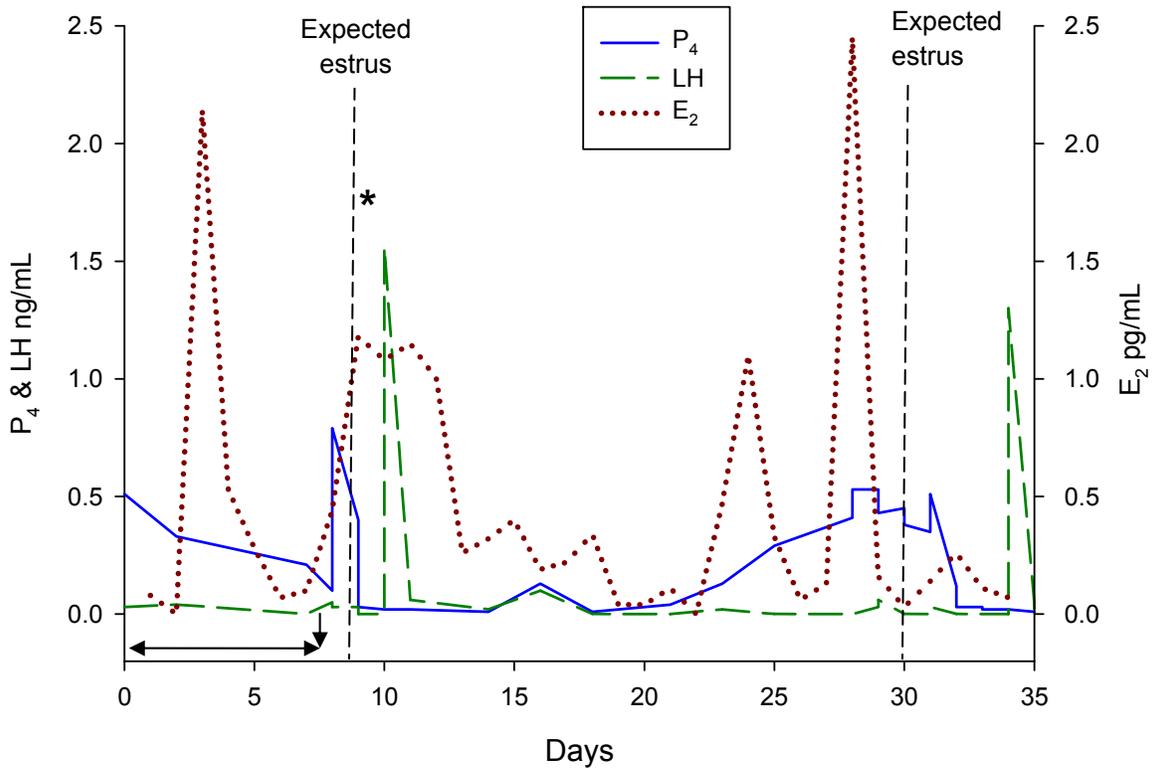


Figure 3.9 The combined hormone profiles for progesterone, estrogen and LH for animal #259 during Regimen 2 (altrenogest protocol). In this particular animal two small LH peaks were observed peri suspected ovulation. Ovulation (*) was suspected to have occurred following induction (day 9; 9 mm), mounting data is insufficient for this animal though she was observed as mounting another female repeatedly. Vertical arrow indicates PGF_{2α} administration on day 7, horizontal indicates altrenogest administration days 0 through 7.

Female #140 Regimen 1

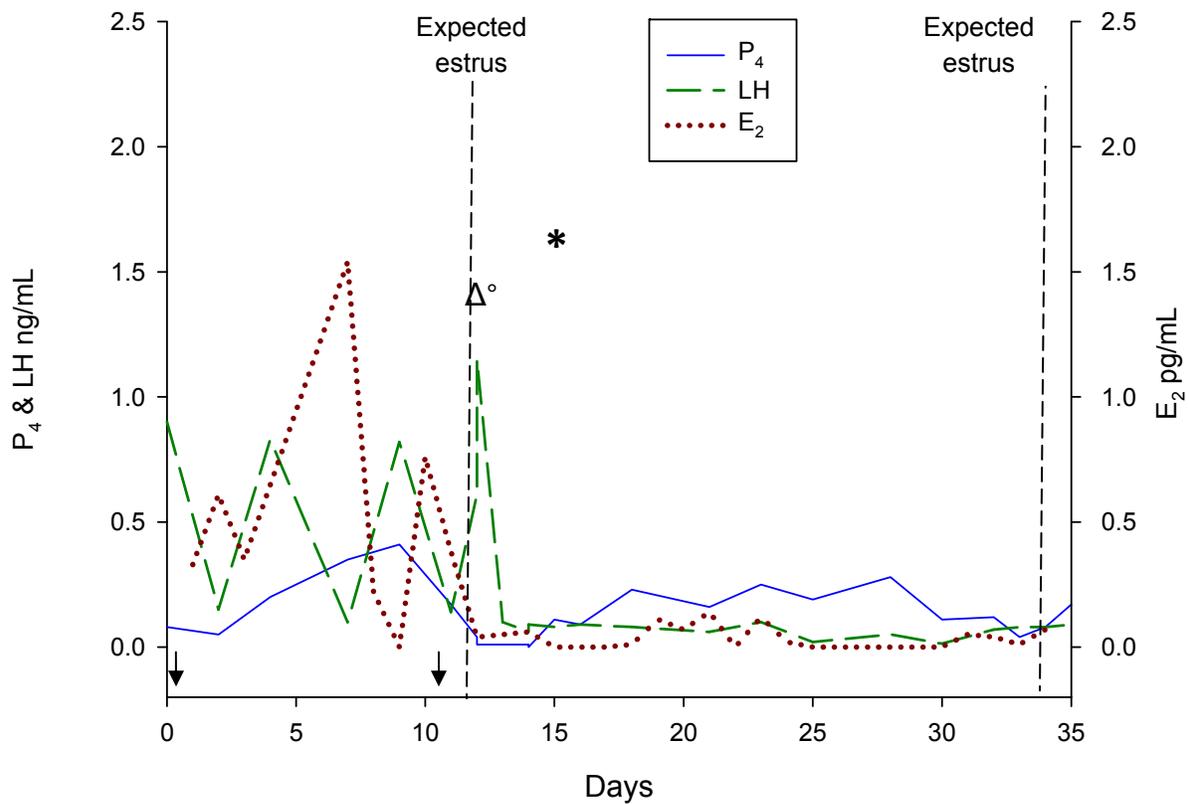


Figure 3.10 The combined hormone profiles for progesterone, estrogen and LH for animal #140 during Regimen 1 (Lutalyse protocol). Ovulation (*) was suspected to have occurred following induction (day 15; 10 mm) and standing estrus (Δ°) was observed on day 12. Arrows indicate PGF_{2α} administration on days 0 and 11.

Female #159 Regimen 1

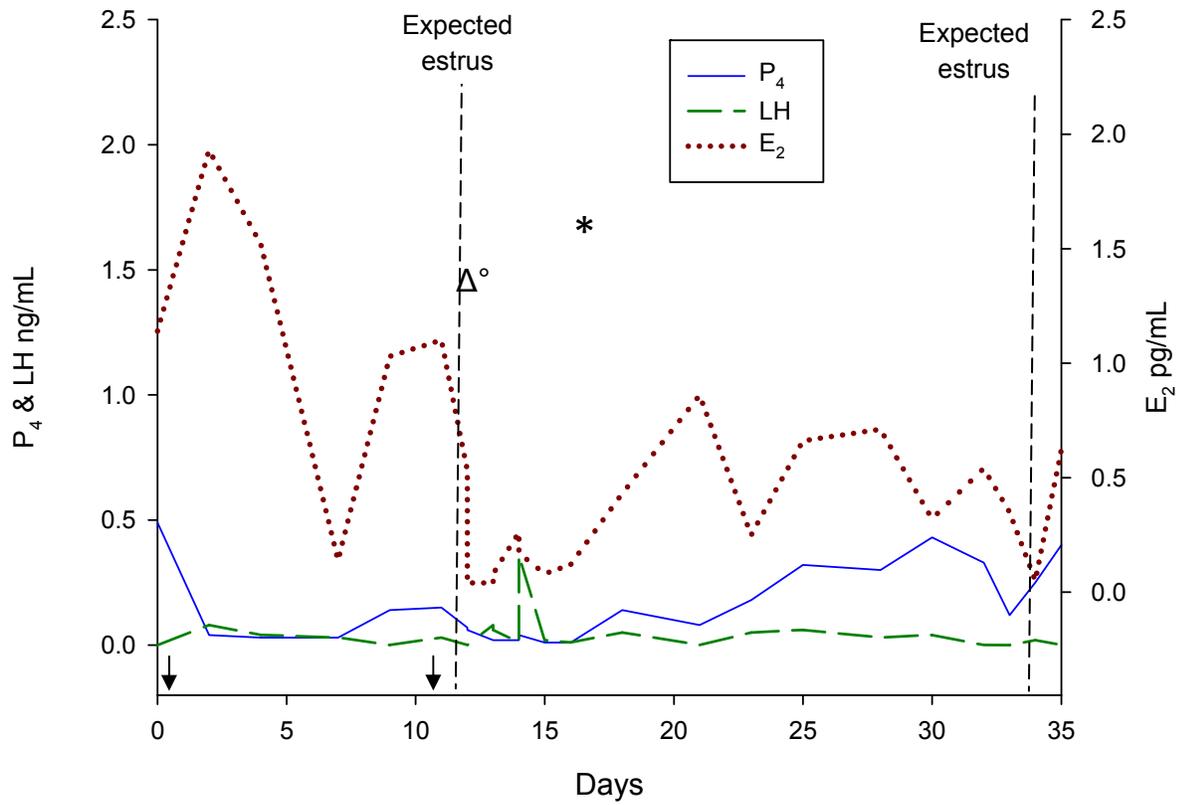


Figure 3.11 The combined hormone profiles for progesterone, estrogen and LH for animal #159 during Regimen 1 (Lutalyse protocol). Ovulation (*) was suspected to have occurred following induction (day 16; 10 mm) and standing estrus (Δ°) was observed on day 11. Arrows indicate PGF_{2 α} administration on days 0 and 11.

within different animals and on different regimens, but both were detected during the natural (second) estrus. There was a trend for elevated estrogen prior to suspected ovulation in 6 of the 7 (86%) ovulations illustrated.

Discussion

The behavioral mounting data indicates that the common eland is least reproductively active during the late morning hours, 6 am to 12 pm. During all other times of the day number of mounts recorded were similar. While mounts were recorded via the HeatWatch system, all were under 2 seconds; this may suggest that the common eland does not display standing estrus strongly when housed in a bachelorette setting. The ability to detect heat by homosexual mounting activity in domestic bovine may be a product of domestication and intensity of display of estrus may reduce vulnerability to predators in nondomestic ungulates (Loskutoff et al., 1995). In addition, the eland may dedicate the morning hours to foraging in its native habitat. For this captive situation however, the decrease in mounting activity may be due to the fact that there are usually keepers present for some time each morning for feeding and cleaning the enclosures.

Other factors that have not been approached in this study, such as male influence on common eland estrus, need to be considered for future studies. Male presence induces early pubertal onset in ewes and gilts (also known as the 'Ram Effect' and 'Boar Effect') and a similar phenomenon is exhibited in the Arabian oryx (Senger, 1997; Blanvillain et al., 1997). Also, male presence has been shown to induce estrus in the Arabian oryx regardless of lactation of postpartum cows (Sempere et al., 1996). The male eland has been indicated to have seasonal rises in corticosterone by fecal assay (Roth et al., 1997; Berg et al., 2008). The seasonality of males may have some effect on the induction and display of estrus in females when housed together or in a non-captive setting.

Regardless of any other factors that have not been assessed here, the common eland will display homosexual mounting activity in a bachelorette herd. In addition to mounting behavior, a female flehmen response was observed on several occasions, as well as, head butting and close following of another individual expected to be in estrus. The flehmen response is also exhibited by a variety of animals apart from domestic species such as the sable antelope, elephants and buffalo (*Bubalus bubalis*) (Rasmussen et al., 1982; Thompson et al., 1995b; Rajanarayanan and Archunan, 2004)

Care must be taken when determining estrus and mounting should not be used as a sole source of identification for eland. Mounting is easily missed and remains a problem for estrus detection in commercial settings such as dairy operations. Various factors, such as temperature and stress, can impede the display of behavioral estrus without preventing ovulation; this 'silent heat' remains a problem for commercial producers as well. The HeatWatch system has improved the efficiency of estrus detection greatly for cattle. The ability to use HeatWatch in this project proved its usefulness for future efforts, but the common eland appears to be a species that does not show strong standing estrus when compared with other bovids (e.g., domestic cattle).

Another species that does not show strong estrus by behavior is the scimitar-horned oryx (Schiewe, 1991b) and the use of scratch off patches was not successful due to the ability of the oryx to reach and scratch the patches with their horns. The attempted use of scratch off patches to detect mounting here was not successful either due to the additive effect of mounting or the lack of scratch off removal due to low mounting duration. HeatWatch proved effective through the ability to detect mounts of less than 2 seconds. Other methods should be included when determining estrus in the eland such as ultrasonography to observe preovulatory sized follicles or identification with a vasectomized male as a behavioral indicator.

Ultrasonography is either incredibly difficult or impossible in most other antelope species, but the common eland is large enough to perform rectal manipulation and reliably view the ovaries via ultrasound. The images that were recorded are some of the first of their kind in an antelope species and show similarities to their domestic cattle counterparts. Ultrasonography has been achieved in the wood bison previously (Othen et al., 1999) which recorded follicular sizes of up to 10 mm. The maximum follicular diameter consistently found in our study was ~10 mm, indicating that this is the ovulatory size of the common eland follicle. While definable corpora lutea were not visualized, large follicles (7 to 10 mm) that disappeared within 24 hours of previous observation were suspected to have ovulated. Ovulations were detected as frequently on the left ovary as on the right (3:4, right:left, respectively), indicating that both ovaries of the common eland are functional and routinely ovulate. This is unlike the sable antelope which only ovulates from the right ovary. Duration of inter-ovulatory interval agrees with previous behavioral observations and thus, the common eland estrous cycle length is 21 ± 1.6 days, similar to that of domestic cattle as well as other antelope species such as lesser kudu, dik-dik, suni antelope, Jackson's hartebeest and bongo (Loskutoff et al., 1990; Schiewe et al., 1991b; Vahala, 1992; Robeck et al., 1997; Nowak, 1999; Metrione et al., 2008). The mean time from PGF_{2 α} administration to ovulation for induced estrus was 69 hours.

Synchronization and induction of estrus was achieved to an extent in this study and each regimen has its advantages, where regimen 1 requires little interaction with the animals (though it does involve more injections), regimen 2 involves less injections (but animal temperament may be more important for agent administration). This information will be useful when implementing artificial reproductive techniques such as artificial insemination and embryo recovery. The use of artificial insemination is beneficial to conservation biology in several ways: (1) it can be used to circumvent the challenges faced by incompatible pairs and (2) it can alleviate costs and stress associated with moving animals for breeding purposes (Wildt and Wemmer, 1999).

In addition to these characteristics that have been outlined, one of the achievements of this project was the ability to consecutively rectally palpate and ultrasound the common eland ovaries and reproductive tract without chemical immobilization or general anesthesia. Only manual restraint, without anesthesia, by hydraulic chute was used for all sessions of blood collection, ultrasound and agent administration. Because the project lasted ~7 months it was determined that the stress imposed was tolerated well enough to not cause mortality in these animals. It should be kept in mind that these particular animals experience personnel in their vicinity daily and have undergone previous studies that required handling within this Tamer[®] chute system (Witru et al., 2004, 2005, 2009) this habituation may have aided in their handling tolerance.

Information is not available as to whether the common eland carries pregnancy preferentially in one uterine horn or the uterine body, like the llama (Senger, 1997) but we suspect they do not as both ovaries routinely ovulated here. The images gained and the ovulatory follicle size determined can be used in the future for artificial insemination (a more accurate estimation of ovulation) as well as embryo transfer to improve chances for success after such procedures. In addition to this, the average time from final PGF_{2α} injection (69 hours) could be used for these purposes. The visualization of dominant follicles confirmed the estrous cycle length of ~21days as previously described (Nowak, 1999).

Overall, the hormone profiles were not as definitive as we had hoped. However, being able to collect blood samples did prove that routine blood collection is possible in a manually restrained common eland. We believe that two LH peaks were found, however, both occurred during a natural estrus in both animals. Sampling was not frequent enough to feasibly catch all LH peaks, but the presence of these indicates that common eland may exhibit an LH peak. More research is needed to conclusively specify the precise hormone pattern of this species. Further research is also needed to determine if such processes as LH peak prior to ovulation

occur. Such research will be difficult however, as more handling of the animal would be required and the risk of stress induced myopathy or injury is increased (Spraker, 1993). This may be one of the most important aspects of this study. Values were low (less than 2 ng/mL of progesterone) across all samples taken from all animals and no complete explanation for this can be provided. Speculation may be that the progestins assayed for in a commercial bovine (domestic) RIA assay kit do not assess for the particular configuration of eland progesterone, it is not known if eland progesterone is unique.

By the end of this project we had shown that repeated manual restraint of this species without sedation in a hydraulic squeeze and lift chute is possible without mortality; and has been previously demonstrated by Wirtu et al. (2009) in eland and Thompson et al. (1995a, b) in sable antelope. Though, future studies should be performed to confirm this as the animals that were used for Wirtu et al. (2004, 2009) were the same as for this study. It was also noted, but not quantified or qualified here, that some animals tended to habituate to the repeated handling while some animals seemed to sensitize. This may indicate that the temperaments of individual animals may lend themselves more readily to handling and manipulation without anesthesia. The use of anesthesia can slow uterine blood flow (Rosen, 1999); this would be detrimental to the use of artificial insemination because this would slow sperm transport through the reproductive tract. Therefore, it is an advantage to restrain these animals without chemical immobilization repeatedly.

Although an animal was injured during the course of this project and had to be removed for recovery, no animals were lost to fatality as a result of the project. The injury of animal #123 was not attributed to repeated handling but rather an incident with chute operation. Also, repeated blood collection and rectal manipulation is possible without noticeable adverse effects. The information gained during the course of this project valuable and useful, but it also demonstrated that projects like this are feasible without animal loss.

Several similarities have been made from the common eland to the domestic cow such as body size, estrous cycle length, homosexual mounting during estrus, induction of estrus and gestation length. These similarities aid in the adaption of techniques from the domestic cow to the common eland such as ultrasonography and assay of hormones from blood samples. Some more advanced techniques have been attempted, such as ultrasound-guided follicle aspiration (Wirtu et al., 2009), proving the ability to adapt techniques. Control of the estrous cycle in nondomestic species can be advantageous when adapting assisted reproductive techniques. The ability to induce estrus was achieved from this study and shows this to be a feasible method for nondomestic species. This has also been achieved previously in species such as the scimitar-horned oryx, sable, guar and fringe eared oryx (Durrant, 1983; Schiewe, 1991b; Godfrey et al., 1991; Thompson and Monfort, 1999) and through estrus control, timing of ovulation can be estimated more accurately. This should be done with care however, as the response to conventional methods used in domestic bovine species is quite variable (Mohr gazelle, Holt et al., 1996; gerenuk, Penfold et al., 2005; common eland, Pope and Luskotuff, 1999; scimitar-horned oryx, Schiewe et al., 1991b; sable, Thompson et al., 1995a, b). Our study has demonstrated a variable response to estrus manipulation as well.

It is important to remember that this project was only conducted for a portion of the year in south Louisiana and future efforts should examine these parameters for a full year to accurately confirm what has been defined here and within this species. Other factors or parameters to be assessed may be vulvar swelling and a more in depth analysis of the HeatWatch system for efficient use detecting estrus in eland. Consideration of use of the CIDR for estrus synchronization should be included in future studies as it is a staple in commercial cattle industries. In any case, this study has shown that the eland estrous cycle can be manipulated, although with variable responses. Great care should be taken with the implementation of a similar study to a more fractious species as stress remained an issue for this calmer species.

CHAPTER IV

SUMMARY AND CONCLUSIONS

A better understanding of the common eland antelope, and possibly other similar antelope species, has been achieved through the efforts of this study. Characterization of the common eland estrus was the goal and we believe that valuable parameters for future efforts have been defined. This species, native to southern Africa, affords itself to the adaption of assisted reproductive techniques due to its large size and relatively calm nature (Posselt, 1963; Nowak, 1999). Other advantages as a result of this are the ability to rectally manipulate the reproductive tract and repeatedly perform handling procedures without mortality (Wirtu et al., 2009). As with many nondomestic ungulate species, stress is a serious threat that can result in death (Spraker, 1993).

Estrus was characterized through identification of estrual behavior, visualization of follicles and ovaries through ultrasonography, and blood sampling to determine hormone profiles in eight common eland females split into two groups of four. Behavior was observed as homosexual mounting, close following of a particular female and flehmen response. To better detect mounting, HeatWatch patches were applied and mounts were recorded for females in estrus, each mount consisted of 2 seconds or less. Mounts were recorded in similar frequency at all times of the day with the exception of late morning, 6 am to 12 pm. This could be due, however, to the feeding schedule at the ACRES facility, which was within these morning hours or a mechanism to reduce vulnerability to predators. Because the females were housed in a bachelorette herd, the observation of homosexual mounting is important and can be used to help detect estrus. It should not be used as a sole detection method as standing mount times were short in duration (≤ 2 seconds); other methods including ultrasonography and observation of flehmen response should be used in conjunction.

Ultrasonography was performed during estrus and the maximum follicle diameter consistently observed was ~10 mm, determined to be the ovulatory follicle size. Both ovaries of common eland grew follicles of this size, thus we suggest that both ovaries are functional.

Two methods of estrus induction and synchronization were used: The first (Regimen 1) was injection of PGF_{2α} (25 mg) on day 0 and day 11 of the study. The second (Regimen 2) was an altrenogest fed for 7 days (0.22%) at a dose of 2.2 mg (5mL per head per day) and PGF_{2α} given on day 7 (25 mg). We suggest that either protocol could be used for estrus synchronization as numerically estrus induction rates were similar: 2 females ovulated following Regimen 1, and 3 females following Regimen 2 (total 35.7%). Two ovulations were detected during the subsequent natural estrus cycle, totaling seven ovulations suspected of 30 (23.3%). Average time from final PGF_{2α} injection to ovulation was 69 hours. Blood samples were also taken during estrus as well as between estruses. While hormone profiles were not explicit, the fact that sampling was achieved repeatedly without loss is note worthy itself. The parameters that have been defined by this project, in short, are: ovulatory follicle size is ~10 mm in diameter, mean time to ovulation following final PGF_{2α} injection is 69 hours, estrous cycle length is 21 ± 1.6 days, homosexual mounting activity is observed surrounding estrus, both ovaries are functional and estrus can be synchronized and induced with similar methods to those used for domestic cattle.

In addition to these findings, it is noteworthy that repeated handling, sampling and manipulation of these animals was performed without sedation and did not result in mortality, though one female was injured and removed from the project as a result of the chute. Using these parameters, other assisted reproductive techniques can be utilized, such as artificial insemination and embryo transfer. Induction of estrus was variable as well as ovulation which agrees with previous data (Durrant 1983; Schiewe, 1991b; Wirtu et al., 2004) and future efforts should attempt to improve these aspects.

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APPENDIX A ASSAY PROTOCOLS

A1: Progesterone RIA Assay Protocol

The specific assay protocol for progesterone is given according to Louisiana State University standard protocol. Samples were extracted with acetone.

Because samples are from a ruminant, all unknown samples must be extracted with acetone prior to proceeding with the assay.

Extraction: add 20 μ L of unknown sample in duplicate into tubes, add 1 mL of acetone to tubes and centrifuge at 3000 rpm for 10 minutes. Decant supernatant into second set of labeled tubes.

Evaporate acetone in tubes and proceed with assay.

Day 1

- 1 Label all tubes in duplicate
- 2 Add 180 μ L PBSG into all tubes with cornwall pipettor except Total Count (TC) tubes
- 3 Add 20 μ L PBSG into NRS and BC tubes
- 4 Add 20 μ L PBSG to unknown sample tubes
- 5 Add 20 μ L of LE2, HE3 and 1.0 ng/mL STD with single pipettor into appropriate tubes
- 6 With all tubes in numerical order, pipette 250 μ L of 125 I labeled Progesterone (provided in kit) shake gently to mix
- 7 Pipette 250 μ L of first antibody (rabbit anti- progesterone) into all tubes except TC and NRS tubes
- 8 Pipette 250 μ L 1: 420 NRS into NRS labeled tubes. Vortex all tubes to mix
- 9 Incubate tubes at 4°C overnight (at least 12 hours, as long as 48 hours)

Day 2

- 10 250 μ L of precipitating agent (goat anti-rabbit) to all tubes except TC tubes. Incubate tubes at 4°C overnight (at least 12 hours)

Day 3

- 11 Load all tubes into centrifuge carriers and add 1mL of COLD PBS to all tubes. Centrifuge at 3000 rpm for 30 minutes. After centrifuge stops, remove carriers and decant supernatant into radioactive waste receptacle. While still inverted, blot on clean paper towels for 4 to 6 seconds.
- 12 Finally, count all tubes in numerical order in gamma counter for 1 minute.

A2: Estradiol RIA Assay Protocol

The specific assay protocol for estradiol is given according to Louisiana State University standard protocol. Diethyl ether is used for extraction because of the smaller amount being assayed for (pg/mL).

Because samples are from a ruminant, all unknown samples must be extracted with acetone prior to proceeding with the assay.

Extraction: add 20 μ L of unknown sample in duplicate into tubes, add 1 mL of diethyl ether to tubes and centrifuge at 3000 rpm for 10 minutes. Place tubes in freezer overnight (at least 12 hours). Decant supernatant into second set of labeled tubes. Evaporate diethyl ether in tubes and proceed with assay.

Day 1

- 1 Label all tubes in duplicate
- 2 Add 180 μ L PBSG into all tubes with cornwall pipettor except TC tubes
- 3 Add 20 μ L PBSG into NRS and BC tubes
- 4 Add 20 μ L PBSG to unknown sample tubes
- 5 Add 20 μ L of LE2, HE3 and 1.0 ng/mL STD with single pipettor into appropriate tubes
- 6 With all tubes in numerical order, pipette 250 μ L of 125 I labeled Estradiol (provided in kit) vortex to mix
- 7 Pipette 250 μ L of first antibody (rabbit anti- progesterone) into all tubes except TC and NRS tubes
- 8 Pipette 250 μ L 1:420 NRS into NRS labeled tubes. Vortex all tubes to mix
- 9 Incubate tubes at 4°C overnight (at least 12 hours, as long as 48 hours)

Day 2

- 10 250 μ L of precipitating agent (goat anti-rabbit) to all tubes except TC tubes. Incubate tubes at 4°C overnight (at least 12 hours)

Day 3

- 11 Load all tubes into centrifuge carriers and add 1mL of COLD PBS to all tubes. Centrifuge at 3000 rpm for 30 minutes. After centrifuge stops, remove carriers and decant supernatant into radioactive waste receptacle. While still inverted, blot on clean paper towels for 4 to 6 seconds.
- 12 Finally, count all tubes in numerical order in gamma counter for 1 minute.

A3: Luteinizing Hormone (LH) RIA assay protocol

The specific assay protocol as given by Louisiana State University standard protocol. Samples were not extracted as this is a protein assay. The LH assay is "in house" for the LSU RIA lab (Thompson et al., 1983) and depending on the institution adjustments should be made. Commercial kits are available.

Because this is a protein assay, no extraction is necessary.

- 1 Label all tubes in duplicate
- 2 Add 200 μ L of sample is added to each unknown tube
- 3 Add 200 μ L LE2, HE3, 1.0 ng/mL STD to appropriate tubes
- 4 Add 200 μ L 1:420 NRS and 1:425 ARGG to NRS and BC labeled tubes
- 5 Add 200 μ L I^{125} labeled anti-LH hormone to ALL tubes
- 6 Add 500 μ L to all tubes except TC
- 7 Vortex to mix and incubate at 4°C overnight (12 hours)
- 8 Load all tubes into centrifuge carrier and add 1 mL of COLD PBS to all tubes.
Centrifuge at 3000 rpm for 30 minutes. Remove carriers and decant supernatant into radioactive waste receptacle. While still inverted, blot on clean paper towels for 4 to 6 seconds.
- 9 Finally, load into gamma counter in numerical order and count for 1 minute

**APPENDIX B
SUPPLEMENTAL IMAGE**

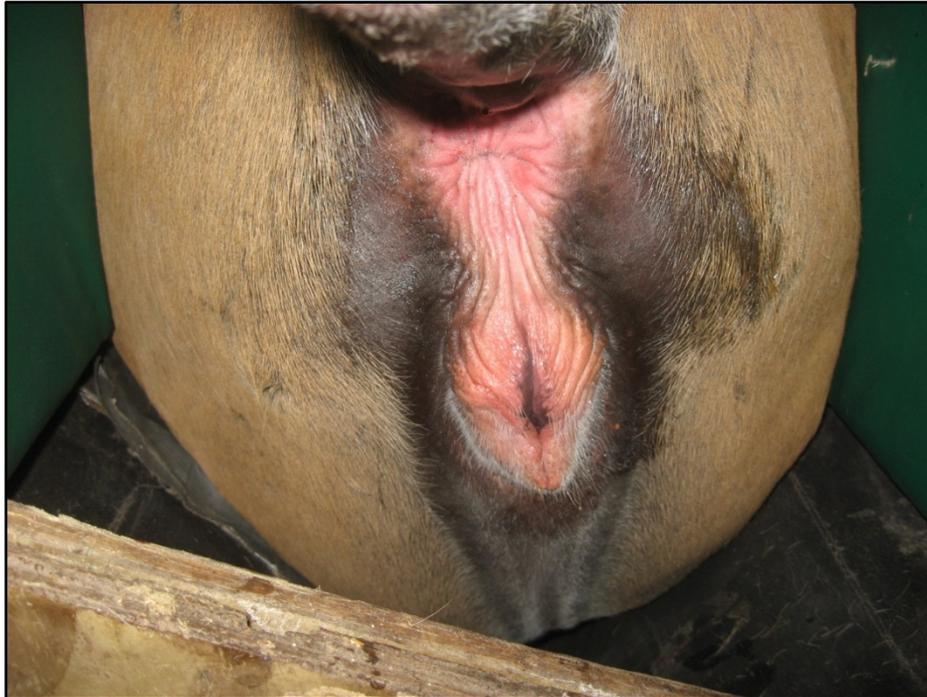


Figure B1. The swollen vulva of a common eland female during estrus while manually restrained for ultrasonography. The swelling of the vulva is an indication of estrus in domestic bovine and may be used as an indication for this species as well, however, more research should be conducted to more accurately assess this phenomenon.

APPENDIX C
SUPPLEMENTAL FIGURES

Female #123 Regimen 1

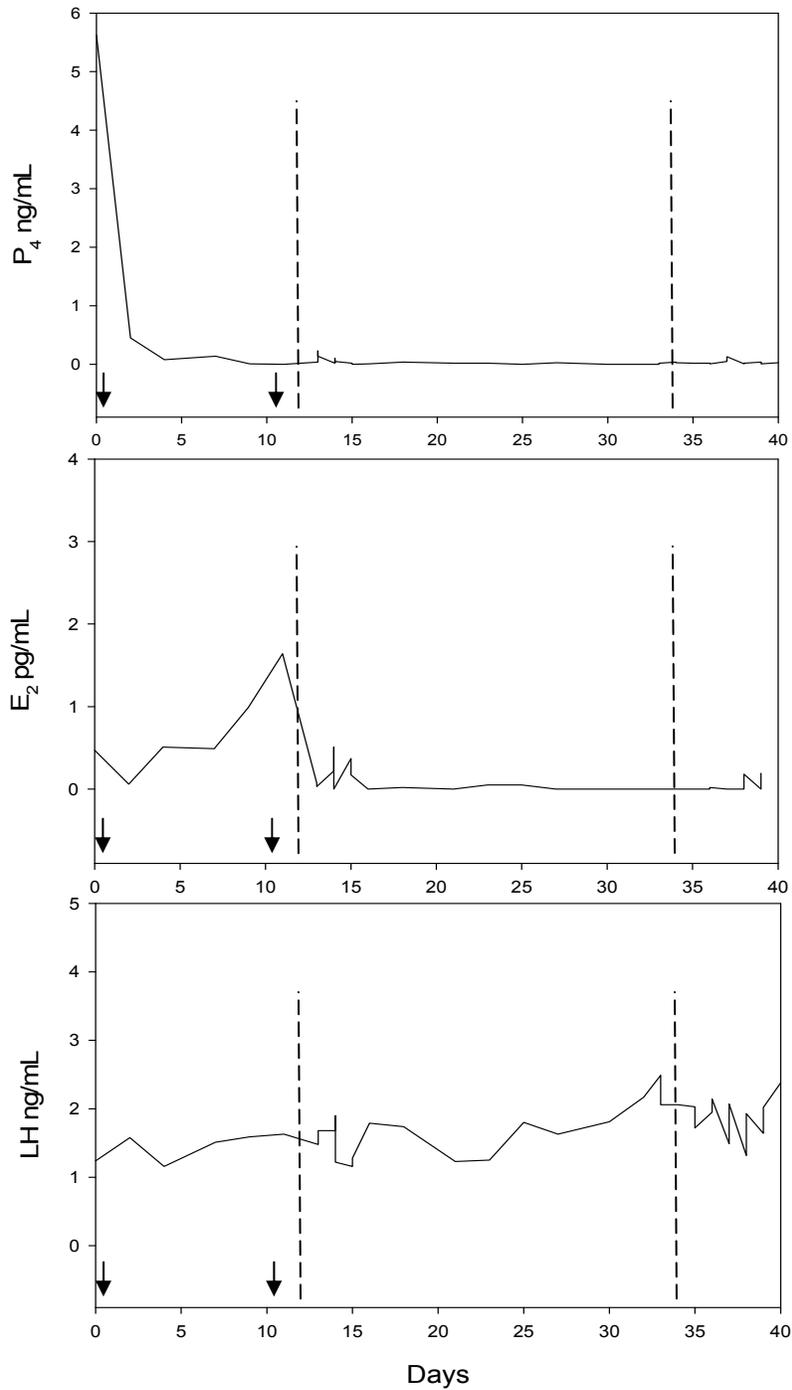


Figure C1 The hormone profiles of progesterone (top), estrogen (middle) and LH (bottom) for animal #123 during Regimen 1 (Lutalyse). Arrows indicate PGF_{2α} administration on day 0 and 11. Vertical dashed line represents expected estrus and initiation of intensive periods.

Female #129 Regimen 1

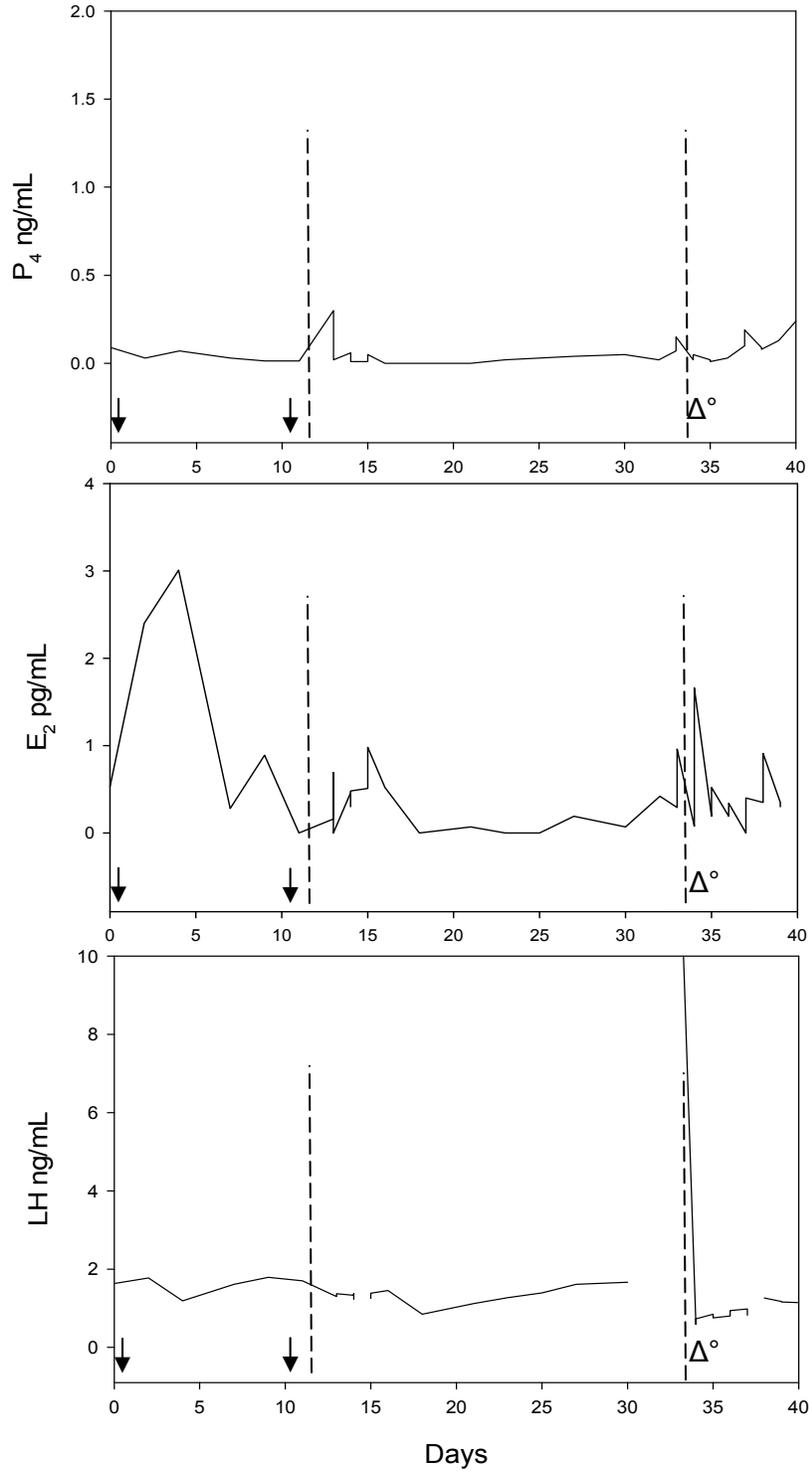


Figure C2 The hormone profiles of progesterone (top), estrogen (middle) and LH (bottom) for animal #129 during Regimen 1 (Lutalyse). Arrows indicate PGF_{2α} administration on day 0 and 11. Vertical dashed line represents expected estrus and initiation of intensive periods.

Female #139 Regimen 1

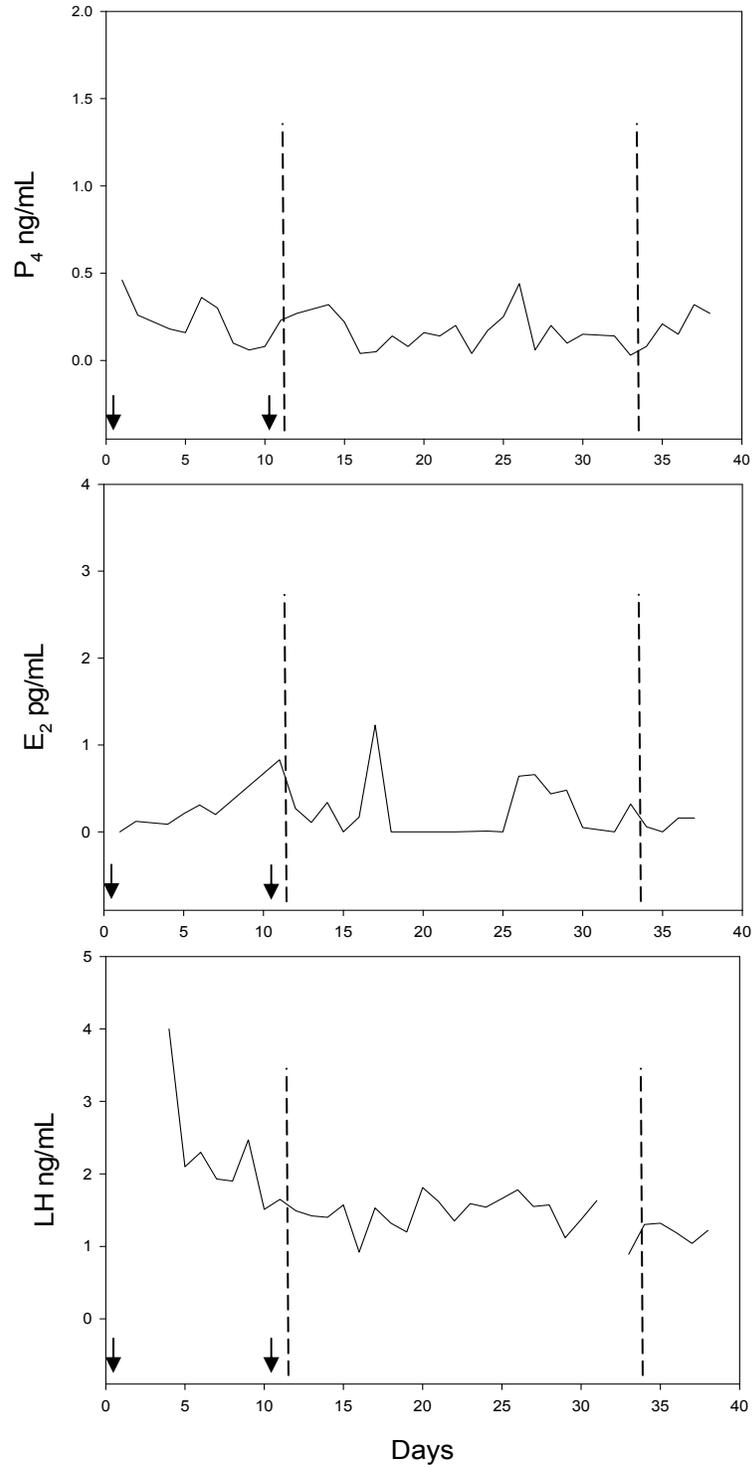


Figure C3 The hormone profiles of progesterone (top), estrogen (middle) and LH (bottom) for animal #139 during Regimen 1 (Lutalyse). Arrows indicate PGF_{2α} administration on day 0 and 11. Vertical dashed line represents expected estrus and initiation of intensive periods.

Female #259 Regimen 1

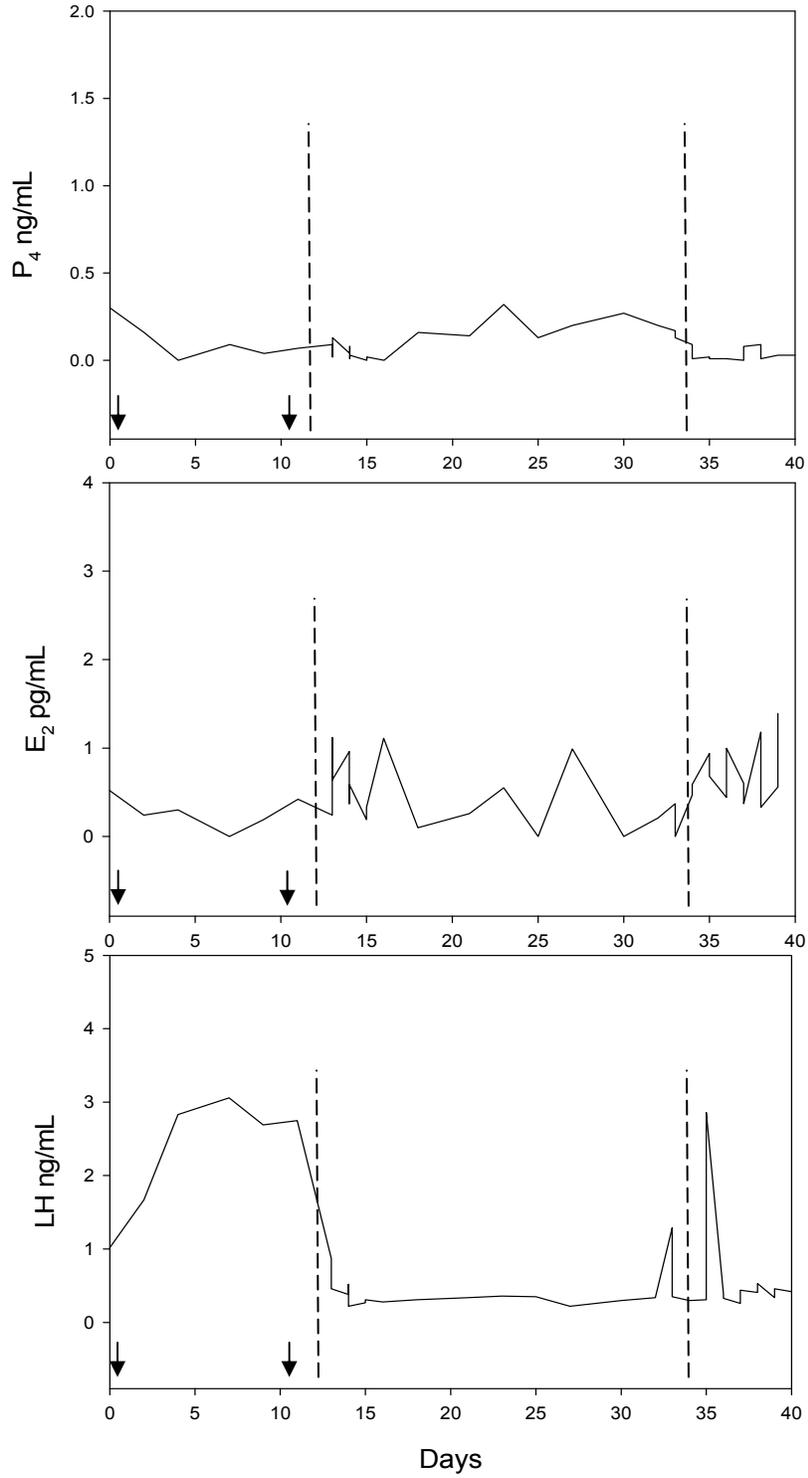


Figure C4 The hormone profiles of progesterone (top), estrogen (middle) and LH (bottom) for animal #259 during Regimen 1 (Lutalyse). Arrows indicate PGF_{2α} administration on day 0 and 11. Vertical dashed line represents expected estrus and initiation of intensive periods.

Female #140 Regimen 2

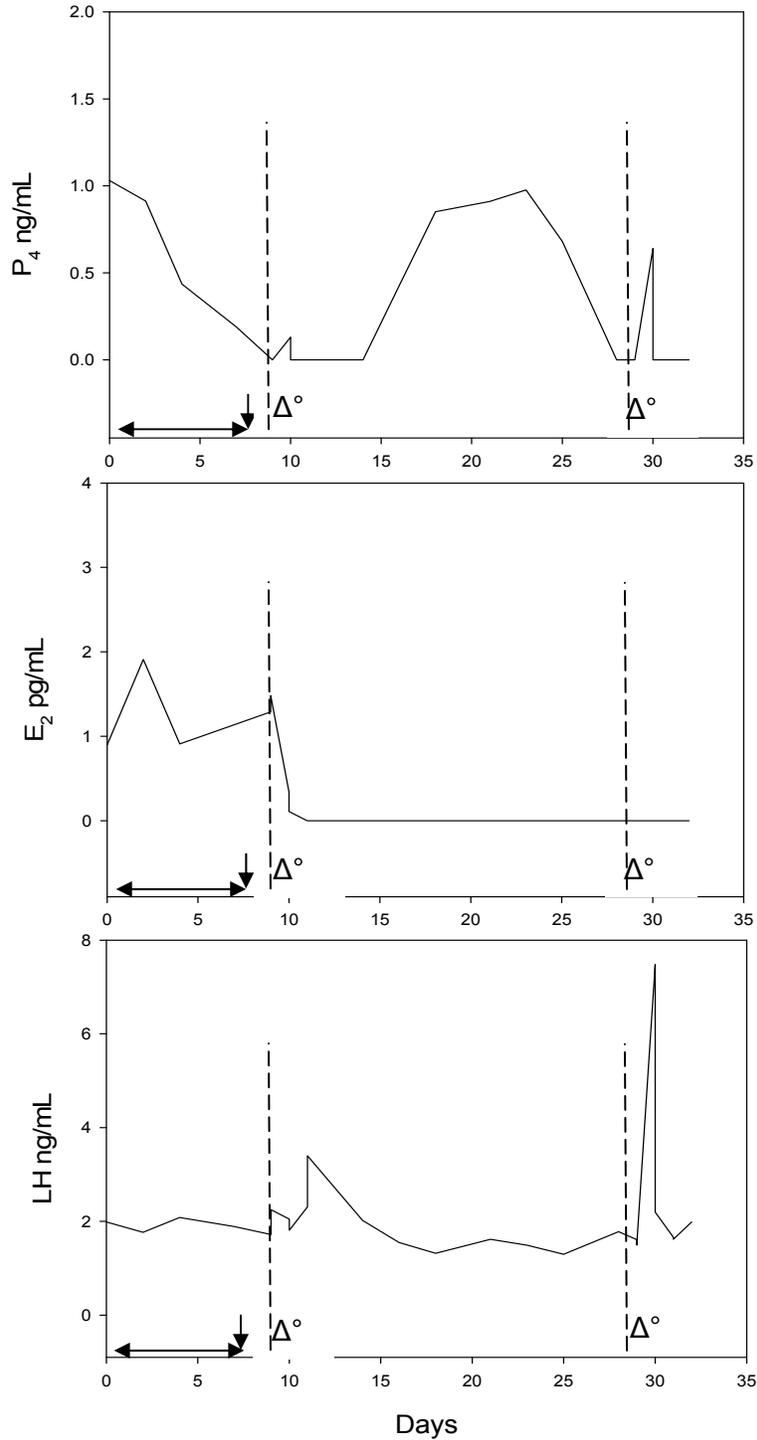


Figure C5 The hormone profiles of progesterone (top), estrogen (middle) and LH (bottom) for animal #140 during Regimen 2 (altrenogest). Vertical arrow indicates PGF_{2α} administration on day 7, horizontal arrow indicates altrenogest administration days 0 through 7. Vertical dashed line represents expected estrus and initiation of intensive periods.

Female #159 Regimen 2

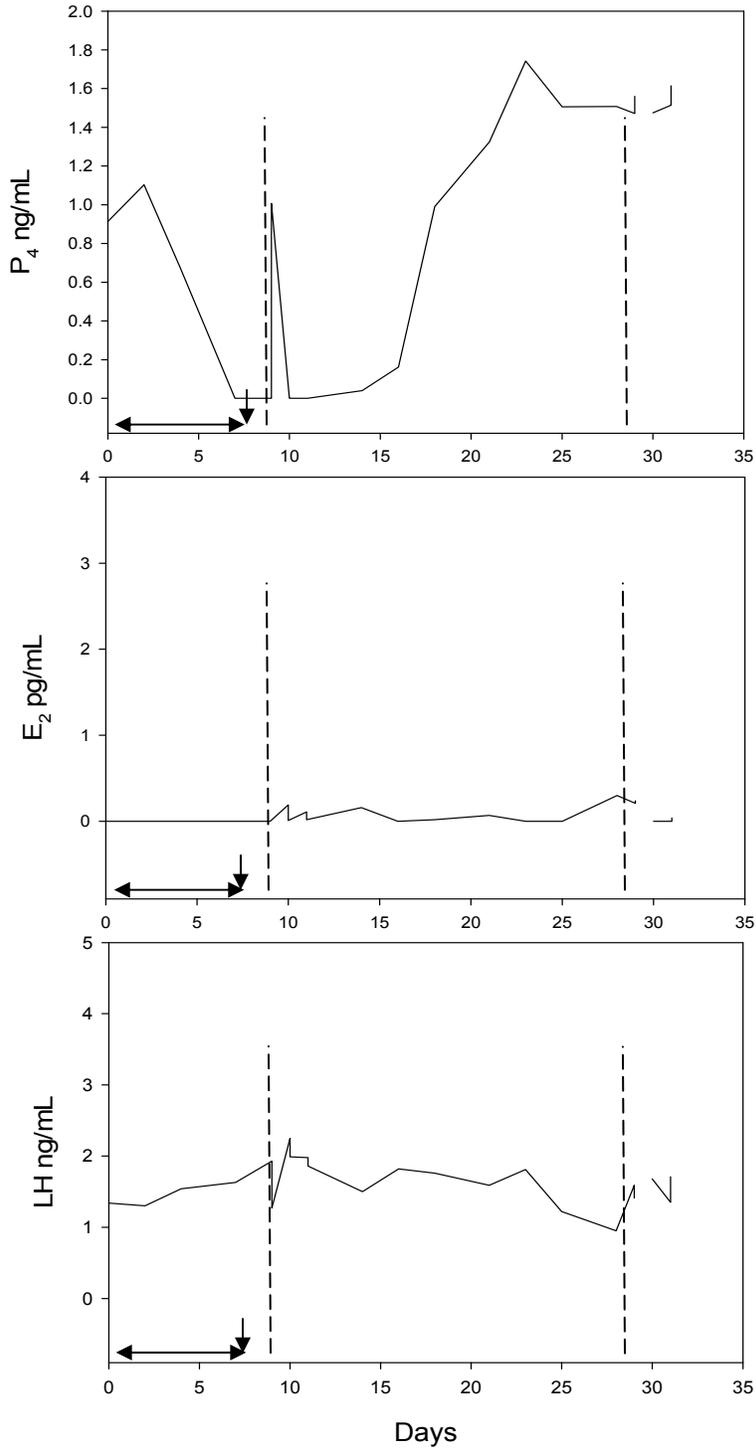


Figure C6 The hormone profiles of progesterone (top), estrogen (middle) and LH (bottom) for animal #159 during Regimen 2 (altrenogest). Vertical arrow indicates PGF_{2α} administration on day 7, horizontal arrow indicates altrenogest administration days 0 through 7. Vertical dashed line represents expected estrus and initiation of intensive periods.

Female #161 Regimen 2

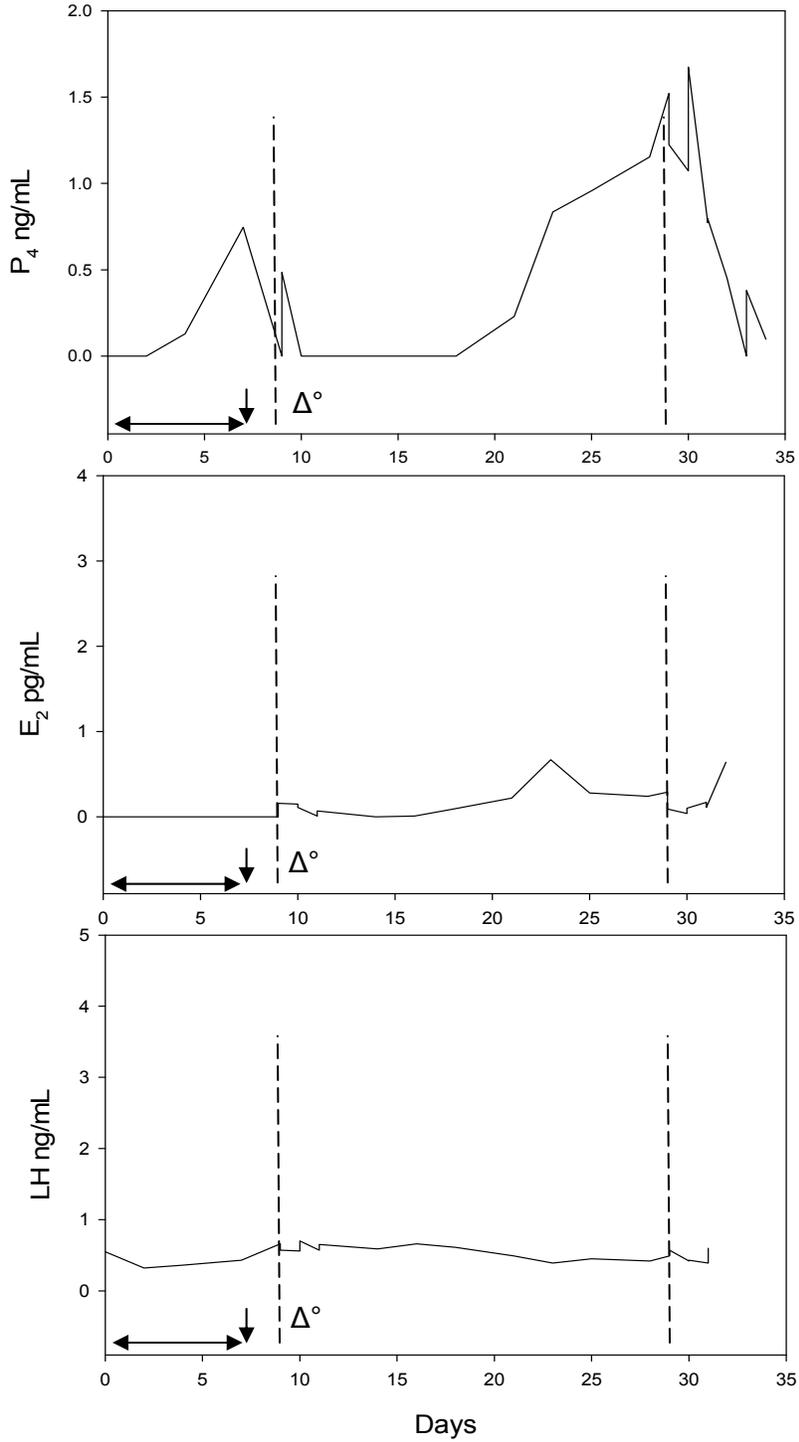


Figure C7 The hormone profiles of progesterone (top), estrogen (middle) and LH (bottom) for animal #161 during Regimen 2 (altrenogest). Vertical arrow indicates PGF_{2 α} administration on day 7, horizontal arrow indicates altrenogest administration days 0 through 7. Vertical dashed line represents expected estrus and initiation of intensive periods.

Female #258 Regimen 2

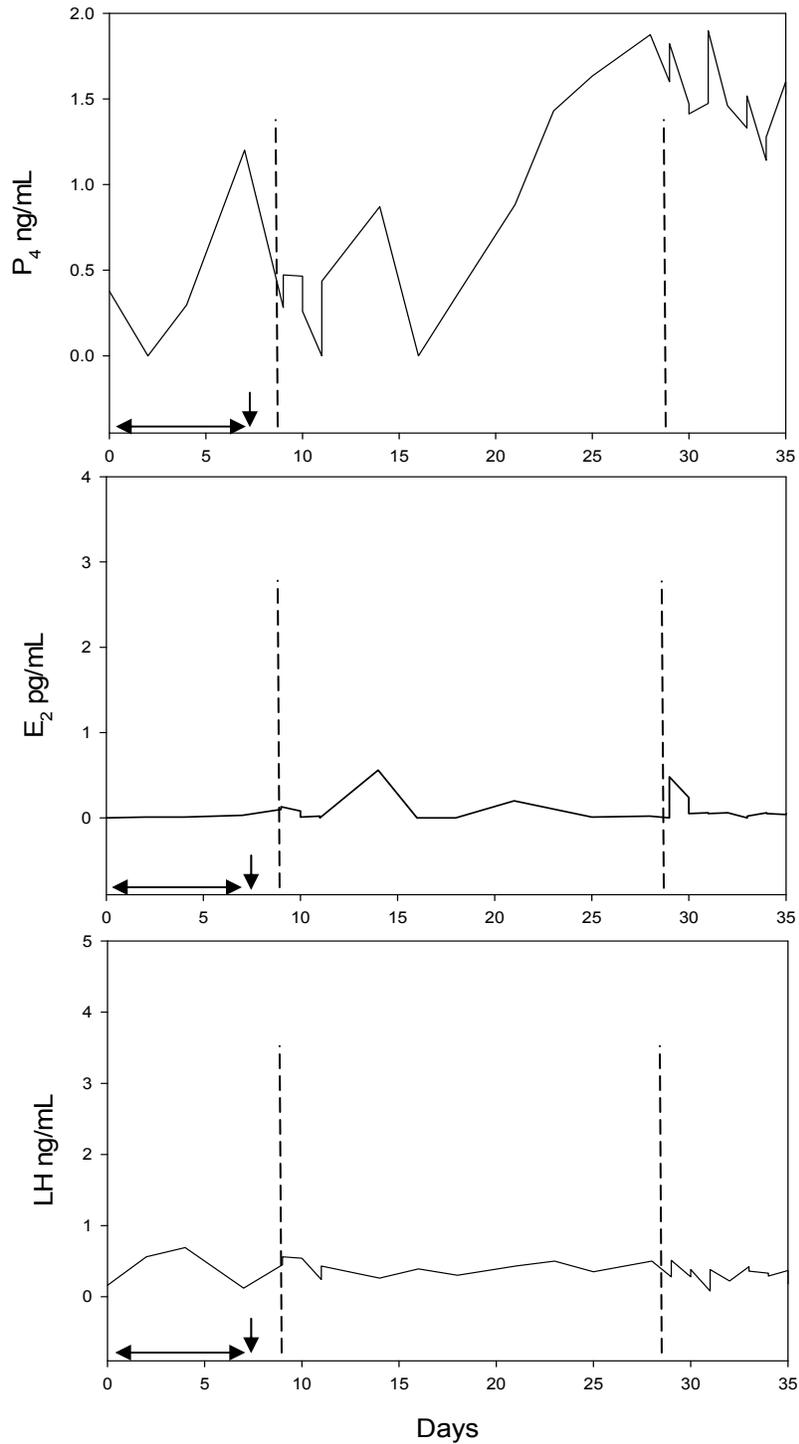


Figure C8 The hormone profiles of progesterone (top), estrogen (middle) and LH (bottom) for animal #258 during Regimen 2 (altrenogest). Vertical arrow indicates PGF_{2α} administration on day 7, horizontal arrow indicates altrenogest administration days 0 through 7. Vertical dashed line represents expected estrus and initiation of intensive periods.

Female #129 Regimen 2

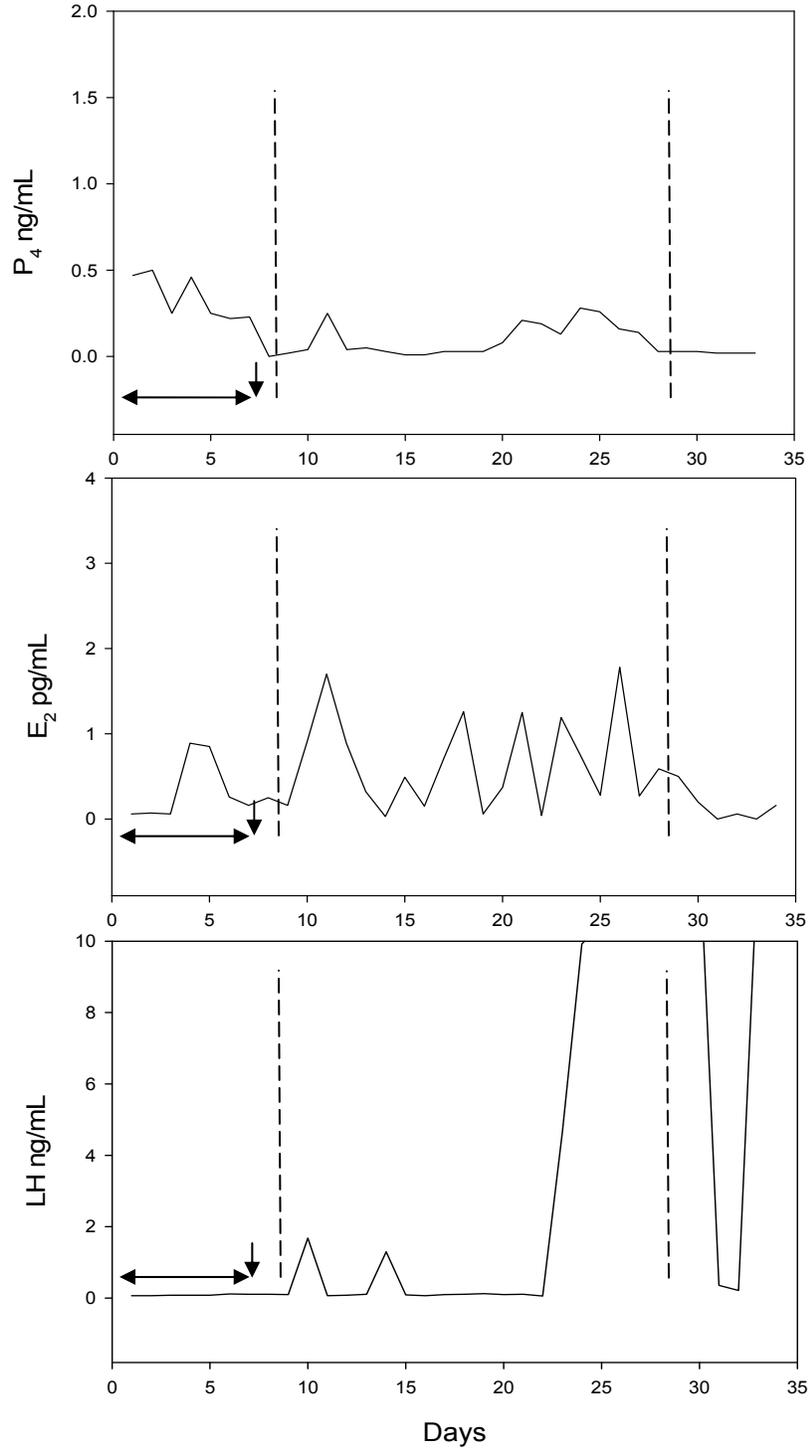


Figure C9 The hormone profiles of progesterone (top), estrogen (middle) and LH (bottom) for animal #129 during Regimen 2 (altrenogest). Vertical arrow indicates PGF_{2α} administration on day 7, horizontal arrow indicates altrenogest administration days 0 through 7. Vertical dashed line represents expected estrus and initiation of intensive periods.

Female #139 Regimen 2

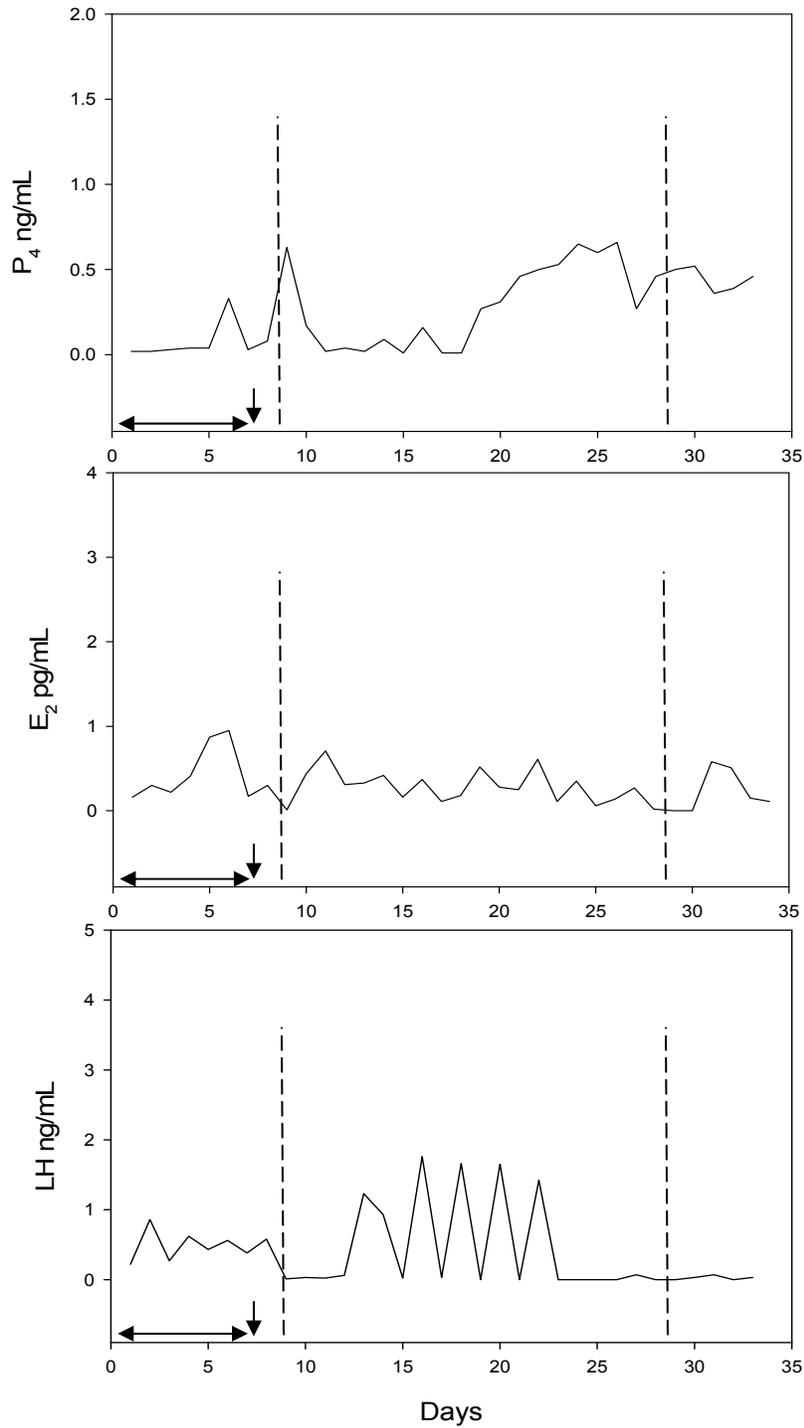


Figure C10 The hormone profiles of progesterone (top), estrogen (middle) and LH (bottom) for animal #139 during Regimen 2 (altrenogest). Vertical arrow indicates PGF_{2α} administration on day 7, horizontal arrow indicates altrenogest administration days 0 through 7. Vertical dashed line represents expected estrus and initiation of intensive periods.

Female #259 Regimen 2

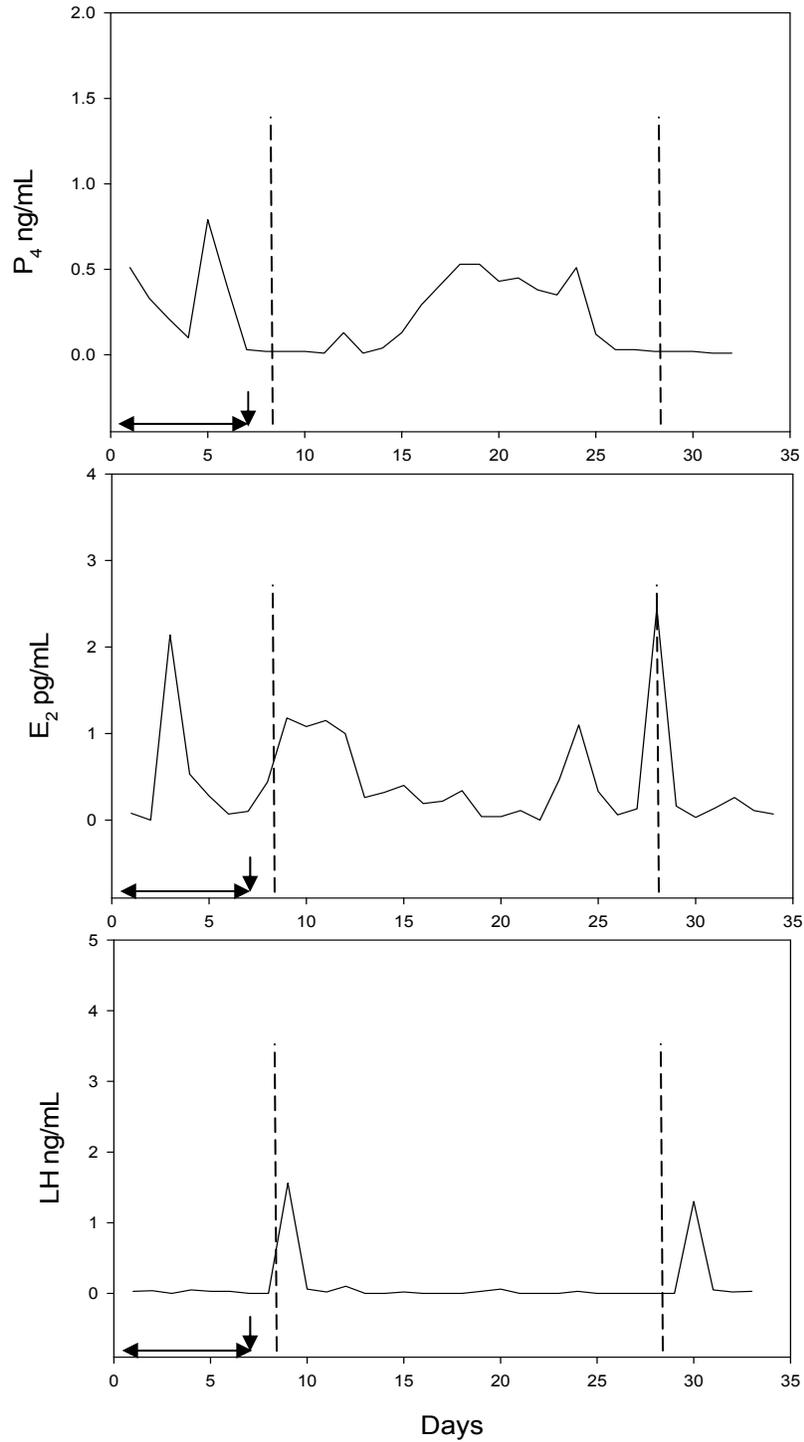


Figure C11 The hormone profiles of progesterone (top), estrogen (middle) and LH (bottom) for animal #259 during Regimen 2 (altrenogest). Vertical arrow indicates PGF_{2α} administration on day 7, horizontal arrow indicates altrenogest administration days 0 through 7. Vertical dashed line represents expected estrus and initiation of intensive periods.

Female #140 Regimen 1

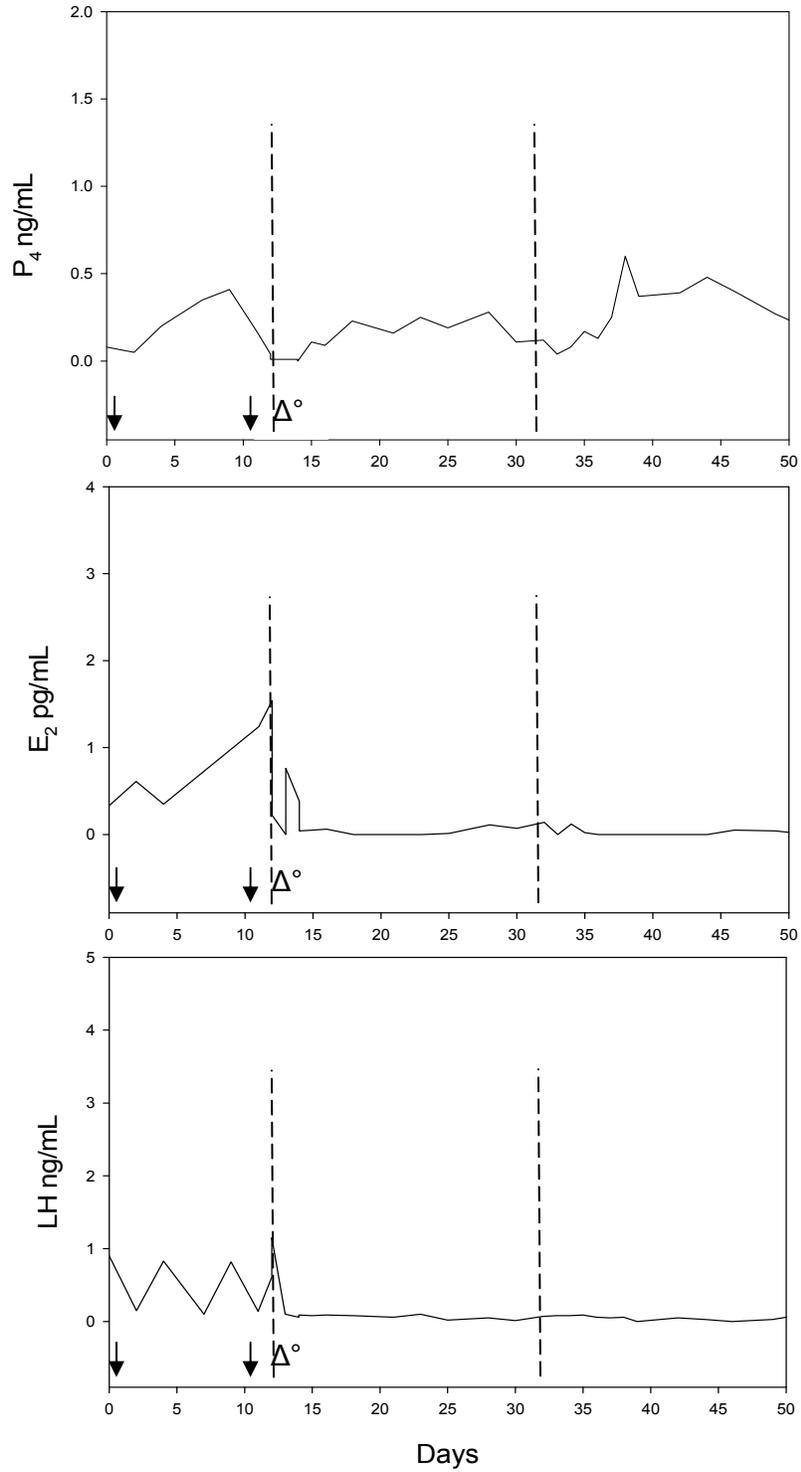


Figure C12 The hormone profiles of progesterone (top), estrogen (middle) and LH (bottom) for animal #140 during Regimen 1 (Lutalyse). Arrows indicate PGF_{2 α} administration on day 0 and 11. Vertical dashed line represents expected estrus and initiation of intensive periods.

Female #161 Regimen 1

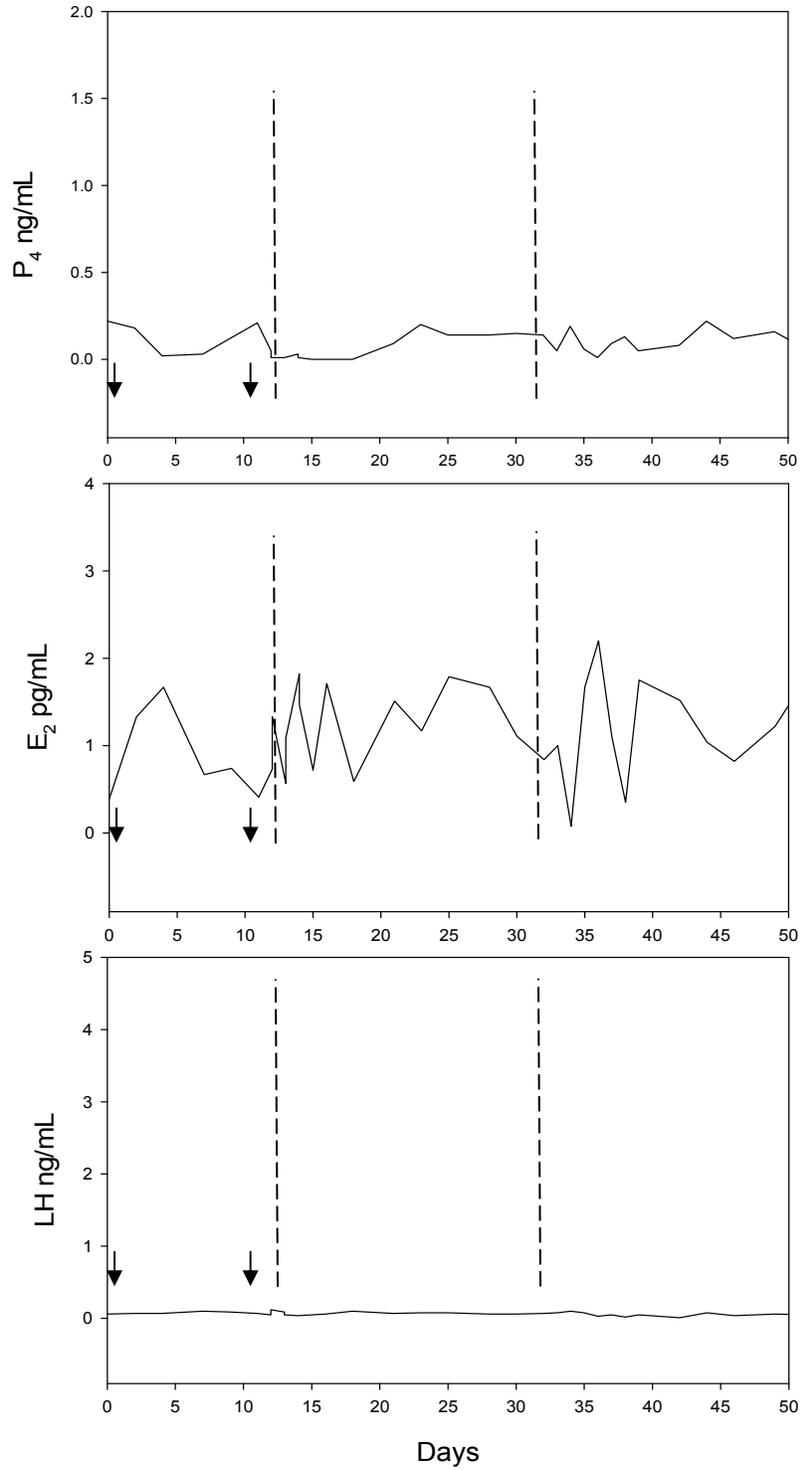


Figure C13 The hormone profiles of progesterone (top), estrogen (middle) and LH (bottom) for animal #161 during Regimen 1 (Lutalyse). Arrows indicate PGF_{2α} administration on day 0 and 11. Vertical dashed line represents expected estrus and initiation of intensive periods.

Female #159 Regimen 1

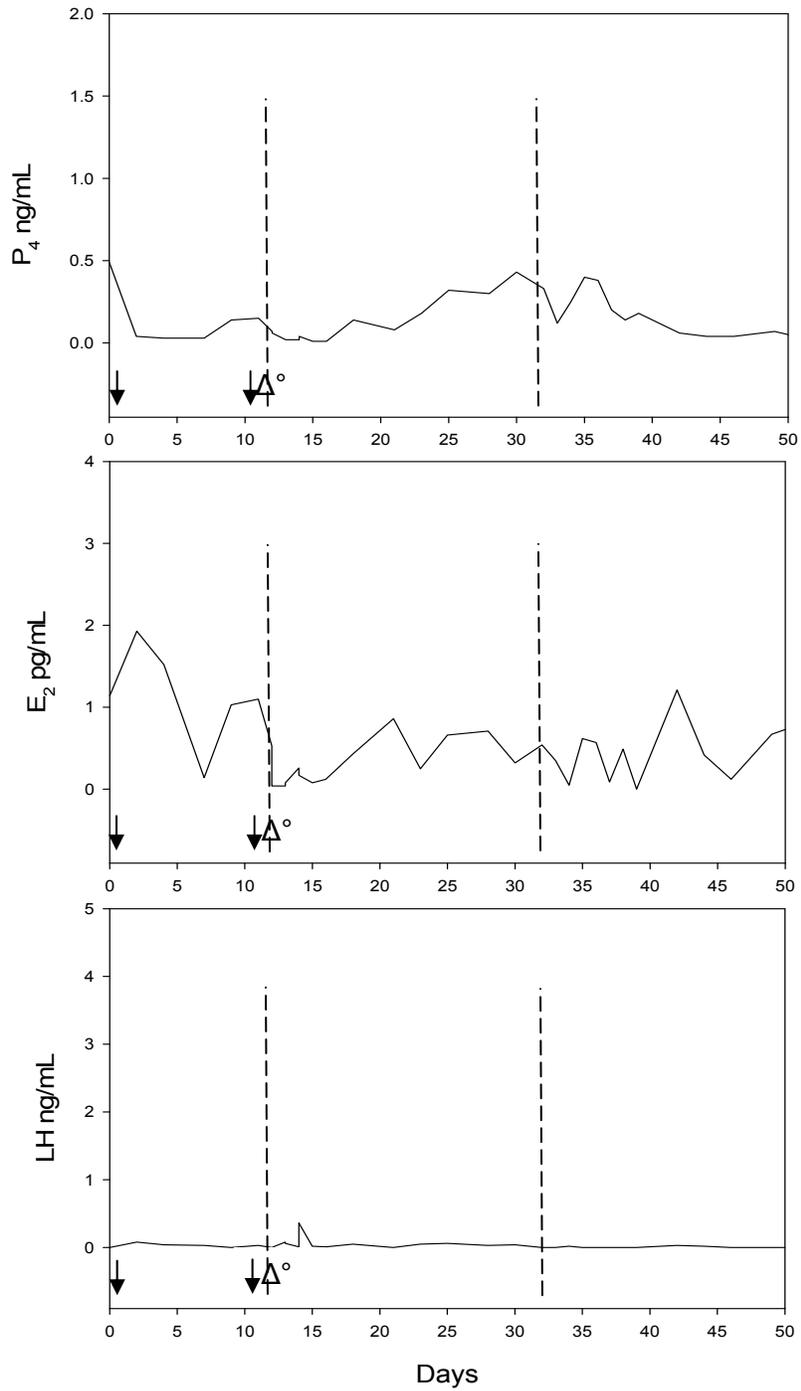


Figure C14 The hormone profiles of progesterone (top), estrogen (middle) and LH (bottom) for animal #159 during Regimen 1 (Lutalyse). Arrows indicate PGF_{2α} administration on day 0 and 11. Vertical dashed line represents expected estrus and initiation of intensive periods.

Female #258 Regimen 1

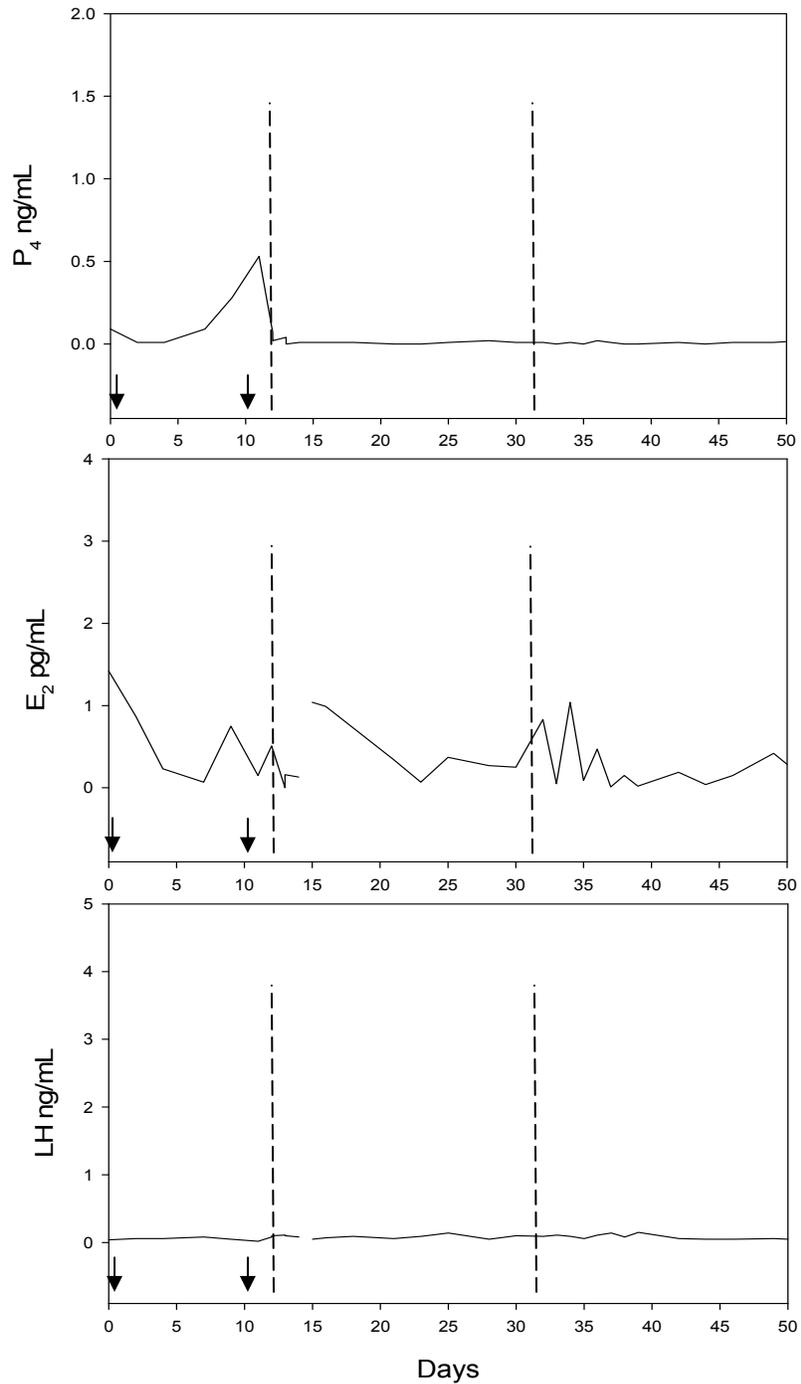


Figure C15 The hormone profiles of progesterone (top), estrogen (middle) and LH (bottom) for animal #258 during Regimen 1 (Lutalyse). Arrows indicate PGF_{2α} administration on day 0 and 11. Vertical dashed line represents expected estrus and initiation of intensive periods.

VITA

Parker Pennington is the only child of Marilyn Mears and Thomas Pennington, born in 1985 in Mobile, Alabama. Her father, Tom, attended Florida State University and received his master's degree in psychology; her mother, Marilyn, attended the University of South Alabama and also received her master's degree in education. Currently, Marilyn is a high school teacher in Alabama and could not be happier with her decision to go back to teaching. Tom is working as a consultant and is enjoying the freedom that he has found with this recent switch of focus.

Parker attended St. Paul's Episcopal school for grades 1 through 12 and graduated in May of 2003. She then attended Louisiana State University for her bachelor's degree, majoring in animal science and minoring in psychology. In addition to undergraduate classes Parker also worked as a student worker at the Louisiana State University School of Veterinary Medicine Teaching Hospital under Dr. Gary Sod in the Farm Animal Clinic. Following completion of her undergraduate degree she decided to attend graduate school. Thankfully Dr. Robert A. Godke allowed her to study under him in the School of Animal Sciences. Her interest in nondomestic species guided her to a research project involving an African antelope species, the common eland (*Taurotragus oryx*) and cooperation with the ACRES facility.